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Canine rabies in Kinshasa, Democratic Republic of the Congo
assessment of maintenance factors and modelling control vaccination schemes

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Canine rabies in Kinshasa, Democratic Republic of the Congo: assessment of maintenance factors and modelling control vaccination schemes

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Dedication

To my beautiful and loving wife, Sarah Ndani Kamba Kazadi

To my lovely daughters Eloise Mulongoy Kazadi and Celine Kamba Kazadi

To my future children

To my good and lovely parents Kalambay Mashinda and Mulongoy Kalumba

To my lovely late parents-in-law Gilbert Ndani Damani and Celine Kamba Bidiki

To my lovely aunts Mujinga Tshimuna and Kapinga Mwa Kalambay

To my beloved late aunts Kamwanya Mwandumba, Mulanga Kalambay and Kamwanya Murima

To my lovely grandmother Kabedi Kalambay

To my beloved late grandfather Gustave Kalambay Mashinda

To my lovely aunts and uncles of Kalambay and Kalumba families

To my lovely sisters and brothers

To Mulunda family

I dedicate this dissertation

I love you all

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Abstract

Introduction

Rabies is a zoonotic disease that causes every year death of 61 000 humans in the world, mainly in Asia and Africa. Dog is the main vector of human rabies. Rabies is a preventable disease. Good dog population management program and regular mass vaccination campaigns have contributed substantially to the elimination of rabies in dog and humans in developed countries. In contrast, rabies remains endemic in majority of resource-poor or developing countries such as the Democratic Republic of the Congo (DRC). This thesis aimed at assessing factors of dog rabies maintenance in Kinshasa (capital of DRC) and at proposing an integrated, efficient and sustainable theoretical rabies control system which is appropriated for resource-poor countries.

Material and methods

Kinshasa, the capital of DRC was selected as the study area. It is the largest city of the country and its population almost equals 11 million people who are potentially exposed to risk of rabies infection. The research was carried out mainly in three peri-urban *communes* of Mont-Ngafula, Ngaliema Lemba where most confirmed dog rabies have been reported. A dog ecology survey was conducted in 22 study sites (*quartiers)* for assessing dog density, dog-keeping practices and rabies vaccination coverage as risk factors of rabies maintenance and establishment of low scale risk maps. It was completed by GPS tracking of a subgroup of owned dogs (n=16) for the characterization of dog roaming behaviour, the street-count for estimation of proportion of feral dogs and the serological evaluation of the immunization status of a subgroup of 132 reported vaccinated dogs (study 1). Thereafter, a risk-based vaccination scheme was developed by considering the canine population dynamics in settings with low risk and high risk for rabies transmission and by considering field serology data regarding efficacy of vaccination and duration of immunity (study 2). Finally, the stakeholder analysis method was used for the description of existing rabies surveillance and control networks in DRC (study 3). It aimed to assess the level of collaboration between them for the efficient control of rabies and to identify in as far as the existing surveillance system might implement the control strategy that emerged from studies 1 and 2.

Results

Study 1 found that the poor dog-keeping practices and the low vaccination coverage were the main risk factors of rabies maintenance in dog population. Between 2 to 100% (mean 60%) of owned dogs were intermittently or continuously free roaming. The absence of a physical barrier appeared as the major reason of the poor restriction of dog movement. However, due probably to poverty, some owners allowed dogs to roam in search of food. Furthermore, irrespective of time since last vaccination, the coverage significantly differed between study sites and ranged from 24 to 81% (mean 56%), whereas the coverage of 40% is the critical threshold under which large rabies outbreaks may occur. Yet, owners' compliance towards mandatory vaccination strongly depended on their socio-economic situation and the subsequent ability to afford vaccination costs. Low vaccination coverage (<40%) occurred almost in areas with low proportions of restricted dogs. The percentage of feral dogs was low (≤2%). The combination of the three main risk factors including dog density, roaming activity and vaccination coverage in form of a risk map reflected the likelihood of rabies transmission at the level of *quartiers*. This risk was found to be high, medium and low respectively in 41% (9/22) and 32% (7/22) and 27% (6/22) of study sites.

Study 2 based on canine population dynamics and field serology evidenced the link between the dog-keeping practices and the turnover rate. The annual turnover rate was 36% in settings with high proportion (≥75%) of free-roaming dogs, whereas it was 17% in settings with low proportion (<25%) of free-roaming dogs. The assessment of vaccine efficacy in terms of serological response to primary or booster vaccination under the field conditions revealed that the efficacy was similar (p=0.24) in puppies (2-3 months: 96%), juvenile (3-12 months: 97%) and adult dogs (>12 months: 100%). By assessing the anamnestic response to booster vaccination, field data provided evidence for a protection of at least 3 years. Thus, given the short life expectancy (≤3 years) of 75% of surveyed dogs, it was hypothesized that vaccine may provide a lifetime protection against rabies for the majority of the investigated dog population. Furthermore, the study found that the systematic vaccination of puppies at 3 months of age was preventing the decrease of the vaccination coverage even in settings with high turnover rate (36%).

Study 3 showed that institutions of wildlife sectors are not included in rabies network, mainly because of the lack of surveillance system of wildlife diseases such as rabies. The rabies network is made up mainly of institutions of medical and veterinary sectors. However,

the collaboration between medical and veterinary sectors was inadequate (weak), notwithstanding the existence of human and animal disease surveillance systems and the countrywide implantation of medical and veterinary institutions. Resources and data are not shared between both sectors either because they are not available or due to lack of legal collaborative framework.

Key findings of above-mentioned studies that are necessary for development of a theoretical framework for improving the efficiency of rabies control in resource-poor settings can be summarized as follows:

- The stratification into risk zones of rabies transmission should allow optimization of resource allocation by initially targeting high risk zone;
- The systematic vaccination of puppies at weaning (≥8 weeks of age) is recommended. It appears as an efficient and cheap method in comparison to annual vaccination of all accessible dogs irrespective of their vaccination status;
- The existing rabies network composed mainly of medical and veterinary structures is an asset for implementing the control strategy that emerged from this research provided that the collaboration between those structures is effective at the operational and strategic levels.

Abbreviations and symbols

ABC	Animal birth control program
BC	Before Christ
B4	Bureau de surveillance
BPSA	Bureau de Production et Santé Animales
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
DCN	Direction de Conservation de la Nature
DEFT	Design effects
DLM	Direction de Lutte contre la Maladie
DNA	Deoxyribonucleic acid
DPSA	Direction de Production et Santé animales
DRC	Democratic Republic of the Congo
FAO	Food and Agriculture Organization
GPS	Global positioning system
HDR	Human to dog ratio
ICCN	Institut Congolais de Conservation de la Nature
IFN	Interferons
INRB	Institut National de Recherche Biomédicale
INS	Institut National des Statistiques
IU	International unit
LVC	Laboratoire Vétérinaire Central
ml	milliliter
NK	Natural killer
OH	One Health
OIE	World Organization for Animal Health
OVCR	Office de vaccination et contrôle de la rage

PEP	Post-exposure prophylaxis
PNS	Peripheral nervous system
RAB	Rabies virus
RFFIT	Rapid fluorescent focus inhibition test
RNA	Ribonucleic acid
SQAV	Service de Quarantaine Animale et Végétale
UNIKIN	Université de Kinshasa
VGP	Vaccine guideline group
WHO	World Health Organization

Chapter 1. General introduction and literature review

1.1. General introduction

1.1.1. Overview on rabies in DRC

Rabies is an acute meningo-encephalitis caused by a virus of the genus *Lyssavirus* . Its existence backs to the antiquity period over which the disease was generally associated with bite by “mad dogs” or “raging dogs”. All mammals including human are susceptible to rabies, therefore making rabies one of the oldest zoonotic disease (Steele, 1975; Neville, 2004). It was estimated in 2010 that rabies is responsible for more than 60,000 annual human deaths across the world, predominantly in Asia and Africa (WHO, 2013).

In Africa, rabies outbreak was reported for the first time in Algeria in 1858 (Steele, 1975). To date, rabies remains a public health threat that kills around 23 800 human per year, mainly through bites of dogs (Knobel et al., 2005; WHO, 2013). However, it should be underlined that this burden of rabies in Africa is an estimate determined in 2010 on the basis of probabilistic methods such as the probability decision- tree approach. Such estimates lack accuracy and need rabies field data for validation (WHO, 2013). In Democratic Republic of the Congo (DRC), rabies was confirmed for the first time in dog and human in 1923 and 1935, respectively (Repetto, 1932; Chesterman and Liégois, 1937). According to published and unpublished laboratory data, more than 1400 dog rabies cases and 2 jackal rabies cases were confirmed across the country by the three national veterinary laboratories in Kisangani, Lubumbashi and Kinshasa, from 1939 to 2017 (Courtois et al., 1964; Makumbu, 1977; Bula and Mafwala, 1988; Twabela et al., 2016). Nowadays, the dog seems to be the main vector of rabies in DRC, given the close physical and spatial relationship between dogs and people. In Kinshasa, about 9% of households owned at least one dog (Kazadi et al.,2020). A similar proportion (10%) of dog-owning households was reported in Matadi, the capital of the Province of Kongo Central (Mbilo et al., 2019). The dog is usually given in the hands of young people who daily take care of him (feeding, cleaning) and which could be considered as the true dog owner rather than the chief of family (personal observation). Leftovers from human consumption are the main source of dog food. Few dogs receive proper housing, husbandry and veterinary care. As is the case in many sub-Saharan African countries, the great importance of owning a dog is given to sociocultural and economic purposes rather than companionship purpose. Accordingly, the dog is kept for guarding, hunting, meat consumption and mystical protection (Makumbu, 1977, Akakpo, 1985, Kazadi et al., 2017, Mbilo et al., 2019; Kazadi et al., 2020).

In DRC, since 1923, large and small rabies outbreaks often occur throughout the country, but they are usually not documented. The only documented rabies outbreak occurred in 2009 during which more than 70 people died. Most of victims were children of less than 15 years who were bitten by rabid dogs, and Kinshasa (the capital of DRC) was the most affected city during this outbreak accounting for more than 70% (52/70) of human deaths (Muyila et al., 2014). Beside this outbreak, from 2003 to 2017, 11,098 human exposures, 27 confirmed canine cases and 154 human clinical rabies cases occurred in Kinshasa (Twabela 2016, unpublished data). Most confirmed dog rabies cases were reported in peri-urban *Communes* of Mont-Ngafula, Ngaliema and Lemba (Fig 1.1). Yet, Kinshasa is a megalopolis that has a population almost equals 11 million people (INS, 2017), who are potentially exposed to dog bites and rabies. By looking at these official data about bite victims and suspect rabies cases, it is very likely that they are under-reported as in many endemic countries such Tanzania, Ethiopia and Bhutan (Cleaveland et al. 2002; Kitala et al., 2002; Hampson et al., 2008; Deresa et al., 2010; Tenzin et al., 2011; Jemberu et al., 2013). It appears that the inefficient surveillance system, poor veterinary infrastructures and limited diagnostic capacity of national veterinary laboratories are the main reasons of underreporting level of animal disease data in DRC (Niang and Denormandie, 2008; Diop et al., 2012; Ministère de la Pêche et de l'Elevage, 2017). In particular, given that rabies is a zoonotic disease, further investigation need to be carried out to evaluate the interaction between institutions or sectors which are assumed to be involved in rabies management in DRC for identifying reasons of data underreporting.

Consequently, the true disease burden is obscured for the above-mentioned reasons, while rabies continues to kill dog and humans mostly in poor communities. In addition, despite the existence of laws regarding the regulation of dog rabies control such as the control of dog movement (Royal Decree of 05 May 1936) and the mandatory vaccination of dogs against rabies (Royal Decree of 01 April 1938), rabies is still maintained in dogs, which in turn infect humans mainly through bites. Therefore, the assessment of factors of dog rabies maintenance, the development of a risk-based vaccination scheme and the assessment of the current situation of rabies network in DRC are the topics addressed in the present thesis.

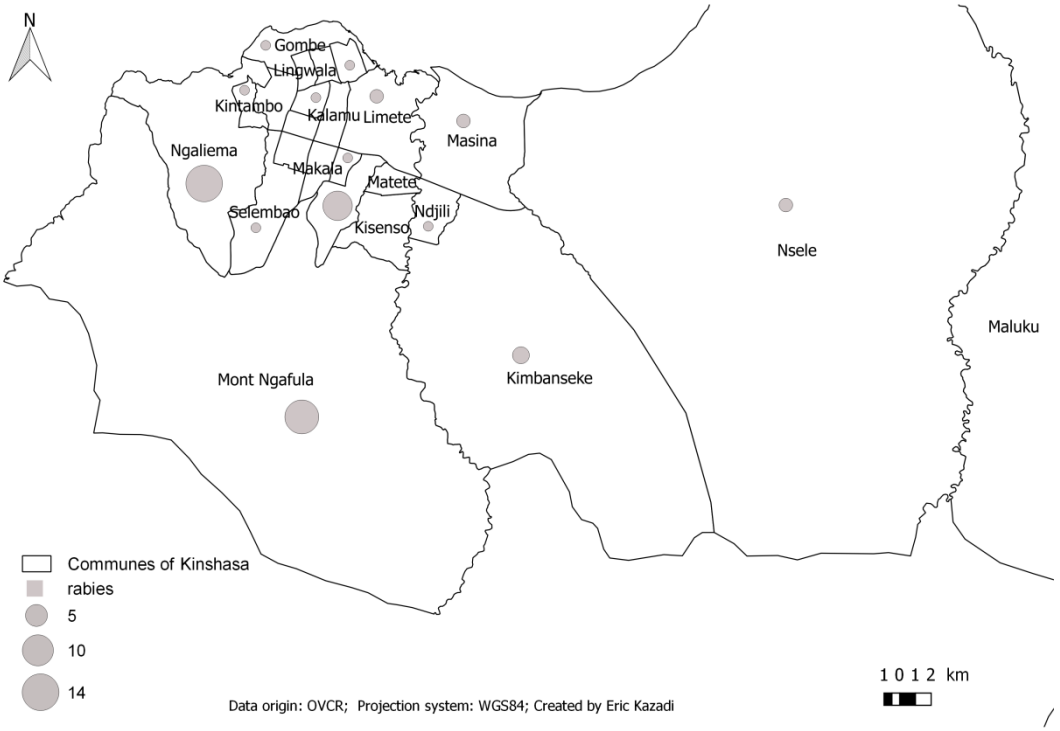

Figure 1.1. Map of the positive dog rabies cases recorded in municipalities of Kinshasa from 2003 to 2017

1.1.2. Thesis objectives

a. General objective

This thesis aimed at assessing factors of dog rabies maintenance in Kinshasa and at proposing an integrated, efficient and sustainable theoretical rabies control program.

b. Specific objectives

Four specific objectives were defined to achieve the main objective as follows:

1. To investigate the risk factors involved in rabies transmission between dogs and to establish a risk map of rabies transmission based on identified risk factors;
2. To develop a risk-based vaccination strategy for rabies control in dog population;
3. To describe the rabies network and to assess the level of interaction between stakeholders;
4. To propose an integrated, efficient and sustainable theoretical rabies control program.

1.1.3. Thesis outline

The present manuscript contains six chapters. Chapter 1 provides the overview on rabies in DRC, the general and specific objectives of thesis and the literature review on rabies by emphasizing on general measures for dog rabies control. Chapter 2 is related to the first specific objective of this thesis. It identifies the main risk factors of rabies maintenance in dog population in Kinshasa, from which rabies transmission risk zones were established using a developed tool for stratification of risk zones. Chapter 3 corresponds to the second specific objective of this thesis. This chapter explores the risk-based vaccination strategies in connection with the established rabies risk zones, the dog population parameters (age structure, turnover rate) and the vaccine characteristics (success rate in puppies, duration of immunity). Chapter 4 is related to the third specific objective. It describes the existing rabies network in DRC and assesses the level of interaction between stakeholders or institutions. Chapter 5 summarizes and discusses the key findings obtained from chapters 2, 3 and 4. It focuses on improving dog rabies control in resource-poor settings by suggesting a theoretical control framework. Finally, the Chapter 6 corresponds to conclusion and perspectives.

1.2. Literature review

1.2.1. History of rabies

Rabies is an ancient disease that backs to antiquity. Human rabies was earlier referenced than dog rabies. The first human rabies case was reported in Egypt and in ancient Greece around 2300 BC, whereas the first canine rabies was described some 500 years BC by Democritus. The saliva from infected dogs was recognised as infective material in 1804 by Zinke. In 1881, Pasteur demonstrated the neurotropism of rabies virus in rabbits and few years later more precisely in 1885, in collaboration with Chamberland and Roux; he discovered the first rabies vaccine which was administered the same year to Joseph Meister, a child aged of 9 years who was attacked by a rabid dog (Steel, 1975; Blancou, 2003).

Despite the discovery of vaccine, the causative agent of rabies was not yet well understood. In 1903, Negri identified inclusion bodies in the brain tissue of rabid dog and humans. He observed that those inclusions bodies were plentiful in the horn of Ammon. He strongly believed that they were the causative agent of rabies and belonged to parasitic protozoan class. In 1927, Sellers developed a useful and widely used method of demonstrating Negri bodies by impression preparations of brain tissue specially stained. In 1935, Webster

and Dawson developed the mouse inoculation test that was the big advance in rabies diagnosis. The rabies virus was observed for the first time on the electronic microscope in 1960. Its structure was completely elucidated 25 years later thanks to molecular biology (Steel, 1975; Bourhy et al., 1993).

As mentioned above, rabies outbreaks were reported since antiquity (Steele, 1975; Neville, 2004). In the DRC, the first dog rabies outbreak was documented in 1923 with laboratory confirmation of rabies in two dogs in Boma city, located in the actual Province of Kongo Central (Repetto, 1932). Then, in 1935, rabies was confirmed for the first time in human, a woman who was bitten by a mad dog in Yakusu, located in the actual Province of Tshopo (Chesterman & Liégois, 1937). The first anatomical pathology laboratory for rabies diagnosis was developed in 1939 by Liégois and was hosted at the veterinary laboratory of Stanleyville (Kisangani) (Courtois et al., 1964). In 1950, dog rabies was officially declared enzootic by national authorities (decree 54/341, 28 September).

1.2.2. Rabies virus

The rabies virus is a bullet shaped enveloped infectious particle (180 nm x 75 nm in size), with a 12 Kb negative sense single-stranded RNA genome and belongs to the genus Lyssavirus of the family Rhabdoviridae and the order Mononegavirale. It has five structural proteins and is surrounded by an envelope in which a glycoprotein (G) is embedded. Besides the G protein, other proteins are nucleoprotein (N), matrix protein (M), non-structural proteins (NS) and RNA-dependent RNA polymerase protein (L) (Woldehiwet, 2005; Van Gucht and Le Roux , 2010). The virus is very sensitive to some environmental factors and it is therefore rapidly destroyed by direct sunlight, desiccation, U.V irradiation, heat, trypsin and common detergents (Awoyomi et al., 2007; Leung et al., 2007).

Speaking to the function of proteins, the N protein plays critical role in viral replication and transcription, as long as it is not phosphorylated (Wu, 2002). The M protein forms oligomers that bind to the outside of the nucleocapsid, giving rigidity to the virion structure and providing a binding platform for the viral glycoprotein and the envelope membrane (WHO, 2013). The G protein is responsible for the induction of protective immunity and contains motifs that define virulence and pathogenicity of the virus. Therefore, any change of G protein (e.g. amino acids) affects the pathogenicity, antigenicity or immunological characteristics of the virus (Cox et al. 1977; Dietzschold et al., 1978; Warrell and Warrell, 2004; Van Gucht and Le Roux , 2010).

The genus *Lyssavirus* is presently composed of 16 species (genotypes) of rabies virus (Table 1.1). The classical *rabies virus* (RABV) and its field strains cause rabies in majority of the cases in humans and animals worldwide, except in Australia and several islands where the virus has not yet been reported. Accordingly, all currently available human and veterinary vaccine strains originate from RABV. The 15 other species are called rabies-related virus, but they may also cause a fatal rabies disease in animals and humans. Rabies-related viruses which are widely distributed in Africa are *Lagos bat virus* (LBV), *Mokola virus* (MOKV) and *Duvenhage virus* (DUVV). Two other rabies-related lyssavirus are country-specific, namely *Shimoni bat virus* (SHIBV) and *Ikoma lyssavirus* (IKOV) discovered respectively in Kenya and in Tanzania. *Australian bat lyssavirus* (ABL) is limited in Australia and can be transmitted from bats to humans and animals. European bat lyssavirus (EBLs 1 and 2) are limited to the Western and Eastern Europe. Seven novel rabies-related lyssavirus have been recovered from bats in Eurasia. These are *Khujand virus* (KHUV), *Aravan virus* (ARAV), *Bokeloh bat virus* (BBLV), *Irkut virus* (IRRKV), *West Caucasian bat virus* (IKOV), *Lleida bat lyssavirus* (LLEBV) and *Gannoruwa bat lyssavirus* (GBLV) (Marston et al., 2012; WHO, 2013, Aréchiga Ceballos et al., 2013; Fisher et al., 2018).

Lyssavirus species are grouped into two distinct phylogenetic groups (phylogroups) and one unclassified group based on serum cross-reactivity against the viral proteins and thresholds in genetic sequence differences (WHO, 2013, Fisher et al., 2018). It is worth noting that the current vaccine strains are originated from the classical rabies virus (RABV), which in turn belongs to phylogroup 1. Laboratory experimental studies reported that besides rabies infection caused by classical rabies virus, the vaccine was poorly effective against infection caused by other rabies species of phylogroup 1 such as Duvenhage virus (Tignor et al., 1977) and European bat lyssaviruses type 1 (Fekadu et al., 1988; Lafon et al., 1988). In addition, the vaccine was ineffective against infection caused by rabies species of phylogroup 2 and unclassified phylogroup (Koprowski et al., 1985; WHO, 2013). It is assumed that the poor or absence of cross-protection provided by the current vaccines against rabies infection caused by other species may be due to differences in the G protein (Bourhy et al., 1993, WHO, 2013).

Table 1.1. Viruses included in the genus *Lyssavirus* (WHO, 2013; Fisher et al., 2018)

Phylogroup	Species (abbreviation)	Primary host	Geographical range
Phylogroup1	*Rabies virus* (RABV)	*Carnivora* and bats (*chiroptera*)	Terrestrial mammals worldwide except in Australia, Antartica, and several islands; bats in the New World only
	Australian bat lyssavirus (ABLV)	bats of *Pteroptus* genus and insectivorous bats *Saccolaimus albiventris*	Australia
	European bat lyssavirus, type1 (EBL1)	Insectivorous bats *Eptesicus serotinus*	Europe, from Spain to the Ukraine
	European bat lyssavirus, type2 (EBL2)	Insectivorous bats *Myotis daubentonii and M. dasycneme*	North-western Europe
	Khujand virus (KHUV)	Insectivorous bat *Myotis mystacinus*	Central Asia
	Aravan virus (ARAV)	Insectivorous bat *Myotis blythi*	Central Asia
	Bokeloh bat lyssavirus (BBLV)	Insectivorous bat *Myotis nattereri*	France, Germany
	Irkut virus (IRKV)	Insectivorous bat *Murina leucogaster*	Eastern Asia
	Duvenhage virus (DUVV)	Insectivorous bats	Sub-Saharan Africa
Phylogroup2	*Lagos bat virus* (LBV)	Pteropodid bats of several genera (e.g.*Eidolon helvum, Rousettus aegyptiacus, Epomophorus spp*.)	Sub-Saharan Africa
	Mokola virus(MOKV)	Unknown	Sub-Saharan Africa
	Shimoni bat virus (SHIBV)	Insectivorous bat *Hipposideros commersoni*	Kenya
Unclassified	*West Caucasian bat virus*(WCBV)	Insectivorous bat *genus Miniopterus*	South-eastern Europe
	Ikoma lyssavirus (IKOV)	African civet *Civettictis civetta*	United Republic of Tanzania
	Lleida bat lyssavirus (LLEBV)	Insectivorous bat *Miniopterus schreibersii*	Spain
	Gannoruwa bat lyssavirus (GBLV)	*Pteropus medius bat*	Sri Lanka, India

1.2.3. Mode of transmission

Since 1804, saliva from rabid dogs was recognized as infective material. Indeed, from the central nervous system of the rabid animal or infected human, the rabies virus reaches the salivary gland and is then excreted in saliva, thereby making the bite as the main route of rabies infection (Singh et al., 2017). The human-to-human transmission through bite is theoretically possible but rare. There is only one report of a 2 year-old boy with encephalitic rabies who bit his mother (Feder et al., 2012), recalling the needs to take protective measures while caring for an infected patient in order to reduce the risk of infection. In contrast, bite is the main route of rabies transmission from animal to animal and from animal to human. In Asian and African countries, the dog bite is responsible for 98% of human rabies cases (Knobel et al., 2005). In addition, the risk of rabies infection by bite (5%-80%) is high as compared to licks or scratches (0.1%–1%) (Hemachudha et al., 2013).

Besides the bite, few non-bite exposures were reported in humans. These are : (i) aerosol transmission during vaccine production as reported by Winkler et al. (1973) or by visiting caves occupied by many bats infected with rabies virus. Although this second way of aerosol transmission remains theoretically possible, it has never been well documented in natural environment, (Gibbons, 2002); (ii) transplantation of tissue or organ. Although this rabies transmission route is rare, in Germany in 2005, a man was infected through corneal transplantation (Hellenbrand et al., 2005); (iii) direct contact of mucosa or fresh skin wounds with infectious material such as saliva (Takayama, 2008), (iv) exposures to live vaccines for animal workers during vaccination (Singh et al., 2017); and finally (v) dog slaughtering process that includes the catching, handling, loading, holding, transportation, keeping in the cages and slaughtering per se. The risk is very high in Asia and Africa where dog meats are consumed, despite it was established that eating of dog meat does not cause the disease (Garba et al., 2013).

1.2.4. Species affected and epidemiological cycles of rabies

All mammals including human are susceptible to rabies, but only a limited number act as reservoirs of lyssavirus. These are members of the order of Carnivora including domestic dogs (*Canis lupus)*, raccoons (*Procyon lotor)*, skunks (*Spilogale putorius)*, foxes (*Vulpes vulpes)*, jackals (*Canis aureus)* and members of the order Chiroptera (bats) (Sedganti and al., 1990). Each rabies variant is maintained in a particular host, notwithstanding the fact that it

can cause rabies in other hosts. Usually the variant die in new host (species) to which it is not adapted and it can only occasionally establish in a new host (Spickler, 2012).

Two main epidemiological rabies cycles are described, namely the urban cycle and the sylvatic cycle. The urban rabies cycle is propagated primarily by dogs, predominantly in Africa, Asia, the Middle East and Latin America. In these regions of the world, more than 95% of human cases are caused by bites of rabies-infected dogs. Rural and poor communities are the most affected (WHO, 1987, Knobel et al., 2005; Spickler, 2012, Global Alliance for Rabies Control, 2015), thereby displaying the contrast with concept of "urban rabies". Although sporadic contamination of dogs by wild animals, the urban rabies cycle has been virtually eliminated in the United States of America, Canada and Europe for several decades. However, the canine rabies variant is apparently established in some wildlife populations (e.g., foxes and skunks in North America) and it can re-establish in dogs from these reservoir hosts (WHO, 1987; Spickler, 2012; Fisher et al., 2018)

The epidemiology of sylvatic rabies cycle is complex given the virus strains, the behaviour of the host species, the ecological and environmental factors. After the elimination of urban rabies, the sylvatic rabies is the predominant cycle in Europe and North America (Spickler, 2012). In Europe, foxes are the main reservoir of rabies since 1950, although the growing role of raccoon dogs (Nyctereutes procyonoides) in the rabies epizootiology (Vitasek, 2004; Van Gucht and Le Roux, 2010; Fisher et al., 2018). In the North America, raccoons stunk and bats are the main wildlife maintenance hosts of rabies. In Latin America, besides coyote, vampire and nonhematophagous bats usually attack the humans and are the main wildlife vectors of human rabies, whereas jackals and mongooses maintain virus in some countries of southern Africa such as Republica of South Africa, Namibia and Zimbabwe (WHO, 1987; Dantas-Torres 2008; Spickler, 2012).

1.2.5. Pathogenesis

The pathogenesis of rabies virus is illustrated in Figure 1.2. Indeed, the rabies virus enters the subcutaneous and muscles tissues of host through a bite wound. Depending on the concentration of the virus inoculated, inoculation site and its density of innervations, the virus remains dormant (eclipse period) at the injected site usually during 3 to 8 weeks, rarely as short as few days (≤1 week) or as long as several years (Greene and Rupprecht, 2006; Heymann, 2008). The incubation or eclipse period is shortened when the bite occurs on the hands, neck, face and head mainly with bleeding, thereby increasing the risk of developing

rabies quickly. In contrast, prolongation of the incubation period gives a chance for post-exposure treatment and immunity for clearance rabies virus in the host (Hemachudha et al., 2002). At the injected site, the virus replicates in muscle tissues and enters the peripheral nervous system (PNS) via nicotinic acetylcholine receptors at the neuromuscular junction (Lentz et al., 1982) and then travels from peripheral nervous system to central nervous system (CNS) through sensory and motor axons via an axonal retrograde transport system at a rate of 12-100 mm/day (Kucera et al., 1985; Kelly and Strick, 2000). When the virus invades the central nervous system, it replicates extensively and causes encephalitis leading to neuronal degeneration and clinical disease develops (Kucera et al., 1985). However, partially due to the genetics of infecting virus, the fatal invasion of central nervous system might not necessarily be accompanied by substantial brain inflammation as seen in some cases of dog rabies (Ugolini, 2011). Finally, the virus runs out through the peripheral nerves and invests in most other organs, especially salivary glands, skin, mucosal surfaces, and gut. The rabies virus causes relatively slow but progressive disease without initial clinical signs which turns into a fatal after disease onset of clinical signs (Jackson, 2010).

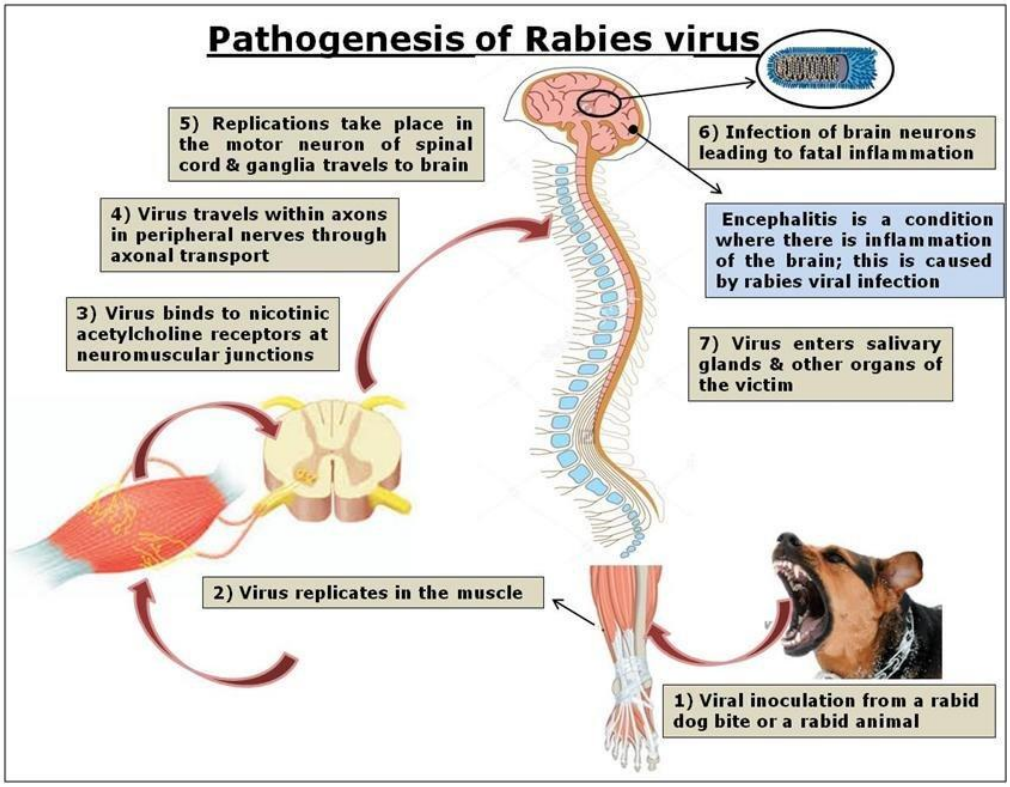

Figure 1.2. Representation of the pathogenesis of rabies virus (Abraham et al., 2017)

1.2.6. Immunology

It is important to note from the outset that in case of infection or vaccination, two categories of immunity responses are triggered for fighting against invading pathogens, namely, the innate and adaptive immune responses. The innate immune response is not pathogen specific and is triggered early, within the first hours following the entry of pathogens or vaccine antigen. It involves the release of cytokines, including type 1 interferons (IFN-*α and -β*) and chemokines, the activation of complement and the attraction of macrophages, neutrophils and natural killer (NK) cells into infected tissues. In contrast, the adaptive (acquired) immune response is very specific to pathogen and requires several days to be set up. It has memory that provides a long-term protection and a quick and efficient response upon re-infection. However, its activation needs information from the innate immune system. Thus, both efficiency of vaccines and defense against pathogens depend upon the robustness of the innate immune responses. When activated, the adaptive response brings into play the cell-mediated immune response, which is carried out by T cells, and the humoral immune response, which is controlled by activated B cells and antibodies (Le Bon and Tough, 2002; Lafon, 2007).

When the rabies virus enters the host through nasal instillation or a breach in the skin and muscles, it triggers an immune response in the periphery before it reaches the nervous system. This includes the secretion of cytokines, the appearance of activated lymphocytes and production of circulating neutralizing antibodies (Lafon, 2007; Singh et al., 2017). Once the rabies virus reaches the nervous system, it escapes the host immune response and protects the infected neurons against apoptosis or premature destruction of neurons. Consequently, both innate and adaptive immune responses are tardily triggered. Neutralising antibody and inflammatory infiltration are usually absent at the time of onset of encephalitic signs. Antibody titres reach substantial levels only in the terminal stages of the disease, which is too late for survival. Cell-mediated immunity plays little role in a rabies infection due to inactivation of T cells by rabies virus (Lafano, 2007; Hunt, 2012; Fisher et al., 2018).

Despite rabies virus escape to immune response, a prompt post-exposure vaccination limits the rabies infection mainly when the incubation period is long (Hunt, 2012). The vaccine induces a sustained antibody response with the help of CD4 T lymphocyte activation. Antibodies can neutralize the rabies virus particles before they reach the neuronal system. This antibody action seems to be the large part of their protective role before the virus enters

the central nervous system. In addition, in the central neuronal system, antibodies can clear rabies virus particles in the early steps of nerve infection (Lafono, 2007). However, T cells are more important for clearance rabies virus from infected tissues than antibodies. Additionally, laboratory studies established that rabies virus is a T-cell-dependent antigen (Turner, 1976; Mifune et al., 1981). T cells induce neuronal apoptosis and thus can initiate an immunopathological reaction for clearance of rabies virus in infected tissues. However, live post-exposure vaccines (DNA and recombinant vaccines virus) induce a strong deleterious CD8 T cell response in the nervous system, which is clinically associated with paralysis. Thus, due to those side effects related to live vaccines, it better to use live vaccines for pre-exposure vaccination regimens because of the robustness of live immunization. In contrast, inactivated post-exposure vaccines that induce mainly B cell activation with the help of CD4 T cells are the most appropriate choice to preserve integrity of the neuronal system (Lodmell and Ewalt, 2001; Lafano, 2007).

1.2.7. Rabies diagnosis

a. Anamnesis and clinical signs

The incubation period of rabies infection varies widely in humans and animals. In most species, the incubation period of natural rabies infection is about 3 weeks but varies from 2 weeks to several months. It may be around 10 days to 6 months in dogs and cats. It seems shorter in unvaccinated animals than in vaccinated animals. (Hudson et al. 1996a , b; Radostits et al., 2007; Spickler, 2012). In humans, the incubation period varies from few days to several years. Typically, it is 1–3 months but may vary from less than 1 week to over a year (Greene and Rupprecht, 2006).

In dogs, the first symptoms of rabies are non-specific and include restlessness, anorexia or an increased appetite, vomiting, slight fever, dilation of the pupils, hyper-reactivity to stimuli, and excessive salivation. Then, the affected dog may become either more aggressive or unusually affectionate, opposite to its normal behaviour and temperament. It may also turn unresponsive to its owner and may prefer sitting in isolation. This stage is called the prodromal phase and usually last for 2–5 days. Once these abnormal behaviours occur, the dog shall be isolated (Campbell and Charlton, 1988; Baer, 1991; Spickler, 2012). The prodromal phase may lead to further progression of the disease in two clinical forms: furious and dumb form. However, the predominance of either clinical forms of rabies is influenced by several factors such as the site of infection, the amount of inoculums and the

source of virus. The virus from vampire bats almost always causes the paralytic form, whereas the street virus causes almost the furious form which is most prevalent in dogs (Radostits et al. 2007). Dog affected by the furious form of rabies becomes quite excited, restless and dangerously aggressive. It bites inanimate objects (own chain, stones, paper, wood, and metal) and attacks other animals and humans. It may not recognize its owner and may show hallucination signs such as snapping at imaginary objects. There may be unusual bark and aimless wandering. Later, there is drooling of saliva due to paralysis of muscles implied in swallowing. Partial paralysis of vocal cords leads to change in tone of bark to howl. In the terminal stage, there is muscular incoordination and paralysis of limbs and trunk. Death occurs within 4 to 8 days after the onset of clinical signs mostly due to respiratory paralysis and convulsions (Spickler, 2012). The dumb form is less common in dogs and is characterized by progressive paralysis. The excitement phase is short or absent in the dumb form. Throat and masseter muscles become paralysed and the animal may be unable to swallow. This causes saliva to accumulate with possible drooling and foaming. There may be facial paralysis or the lower jaw may drop. The dog has a dull or vacant expression and prefers to sit isolated in a corner. It may respond to its owner's call but there is tendency of forgetfulness. Paralysis begins with the muscles of head and neck region. The animal has difficulty in swallowing. This is often mistaken as bone stuck in the mouth and the owner out of ignorance tries to help the dog and gets exposed to infection. General paralysis results in death of the animal usually within 3 to 5 days. However, in dogs, rabies should be differentiated from other neurological diseases such canine distemper, canine encephalitis and poisoning (Campbell and Charlton, 1988; Baer, 1991).

In cats, the furious form is the most common form of rabies. The animal may strike at the air with its forepaws as if it were catching mice. Paralysis of the hind part begins within 2–4 days after the symptoms of excitement, and the animal generally dies in 3–5 days due to convulsions and respiratory paralysis (Radostits et al., 2007).

In ruminants namely cattle, sheep and goats, rabies should be suspected when there is a sudden change in disposition and failure to eat or drink, when the animal becomes paralyzed or runs into objects and when it separates from the herd and stops ruminating (Shultz, 2004). Rabies in ruminants may be manifested either in furious form or in paralytic form. In an experimental study, major clinical findings of the disease included excessive salivation (100 %); behavioural change (100 %); muzzle tremors (80 %); vocalisation (bellowing 70 %); aggression, hyperesthesia, and/or hyperexcitability (70 %); and pharyngeal paralysis (60 %).

The furious form occurred in 70 % cases. The animal usually dies 4–8 days after the onset of the clinical signs (Hudson et al. 1996b; Radostits et al., 2007).

In horses, the clinical signs of rabies included abnormal postures, frequent whinnying, aggressiveness and kicking, biting, colic, lameness, ataxia, paresis of the hindquarters, recumbency, convulsions and terminal paralysis. The furious form occurs in 43 % of cases, some of which began as the dumb form. The death occurs within 5 days after the onset of clinical signs (Radostits et al. 2007).

The clinical findings in pigs are extremely variable and only one or two of the classical findings may occur. Pigs manifest excitement and a tendency to attack or dullness and incoordination. Affected sows show twitching of the nose, rapid chewing movements, excessive salivation, and clonic convulsions. They may walk backwards. There is paralysis in terminal stage and death occurs 12–48 h after the onset of signs (Radostits et al., 2007).

In wild animals, rabies shall be suspected when there is a change in behaviour such as loss of fear of man or unusual friendliness. Nocturnal animals may show abnormal activity during daytime and may attack humans. In the furious form of rabies, there is unprovoked aggression and some animals may attack anything that moves or even inanimate objects. The affected animal may appear disoriented or uncoordinated, or wander aimlessly. It may stumble or fall. Paralysis often begins in the hind legs or throat. Paralysis of the throat muscles can cause the animal to bark, whine, drool, choke, or froth at the mouth. Vocalizations ranging from chattering to shrill scream are observed. Terrestrial mammals such as skunks, raccoons, and foxes usually display furious rabies. Bats often display dumb rabies. They are unable to fly and they may be found on the ground, thereby increasing the risk of infection particularly in children, who are more likely to handle wild animals than adults (Shultz, 2004).

As a reminder, the incubation period of rabies in humans usually ranges between 3 and 8 weeks and is rarely less than 1 week or over 1 year. The length of the incubation period depends on factors such as the amount of virus inoculated the degree of innervation at the site of viral entry, and the proximity of the bite to the central nervous system (Greene and Rupprecht, 2006; Heymann, 2008). The first signs are non-specific and may include itching, pain and paraesthesia at the bite site, headache, anxiety, restlessness, fever and gastrointestinal disorders (Jackson, 2007). When the virus spreads through the central nervous

system, it causes a progressive fatal encephalomyelitis. Both furious and dumb forms may appear, but usually one form predominates. The furious rabies is characterized by irritability, agitation, hyperesthesia, hydrophobia aerophobia, photophobia and generalized flaccid paralysis. Hydrophobia is considered as the pathognomonic symptom of rabies in humans. The affected person shows panic, fear when presented with liquids to drink as a consequence of violent spasm of the gullet and painful laryngospasm (Meslin, 2005; Nigg and Walker, 2009). The furious form of rabies should be differentiated from delirium tremens, botulism, diphtheria, drug ingestion (phenothiazines and amphetamines), plant ingestion (Datura fastuosa), cerebral malaria, whereas the dumb form of rabies that occurs in 30% of human cases, should be differentiated from Guillain-Barre syndrome, polio herpesvirus simiae (Leung et al., 2007; Mallewa et al., 2007, WHO, 2013). It is characterised by generalised paralysis, it runs a less dramatic and usually longer course than the furious form but it is ultimately fatal too (WHO, 2013).

b. Laboratory-based diagnosis

The laboratory-based diagnosis of rabies is usually made after death. Indeed, most diagnostic tests for rabies in animals require brain material which is available only after death (Fooks et al., 2012). Brain smears or touch impressions are used for the detection of virus antigen using the fluorescent antibody test (FAT) or direct fluorescent antibody test (dFAT) both for animals and human samples (WHO, 2013, OIE, 2014). Other diagnostic techniques include reverse transcription polymerase chain reaction (RT-PCR), direct rapid immunohistochemistry test (dRIT) and serological tests such as fluorescent antibody virus neutralization test (FAVN) and rapid fluorescent focus inhibition test (RFFIT) (Lembo et al., 2012; WHO, 2013; OIE, 2014).

Serological tests are rarely used in the diagnosis of rabies. They are used primarily to assess the immune status of man and other animals such dog, cats following vaccination or for assessment of potency of rabies vaccines. They are of limited value in the detection of rabies-infected animals given that the immune responses following natural infection vary considerably, and antibodies may be produced only in the terminal stages (Singh et al., 2017).

In humans, above-mentioned diagnostic techniques can be used to confirm a clinical case of rabies while the patient is still alive, especially when a history of exposure to an animal is lacking or for identification of other people who may have been exposed to the same animal during the public health investigation. Samples are skin biopsy samples or hair

follicles from patients with clinical rabies. However, the intra-vitam diagnosis of rabies in animals is discouraged because of great risk of contamination (WHO, 2013).

1.2.8. Prevention and control of rabies

The present section focuses on prevention of rabies in humans and on measures for rabies control in dog population, given that 98% of human rabies deaths are caused by dog bites in Asia and Africa (Knobel et al., 2005). In addition, even in areas where wildlife species are the rabies reservoir, due to its proximity to the humans, the dog provides a link in transmission between wildlife and humans (Knobel et al., 2007).

a. Prevention of rabies in humans

Rabies is almost without exception a lethal disease. Once neurological signs occur, the lethality reaches 100% in despite of administration of post-exposure prophylaxis. Thus, it is important to prevent rabies before and after suspected or proven exposure to virus by immunization. The pre-exposure prophylaxis is strongly recommended for people, who are at risk for their job such as veterinary and laboratory personnel, their residence or who are travelling in endemic regions. It is mainly based on vaccination (WHO, 2013).

The post-exposure prophylaxis should start as soon as possible after exposure to virus and consists of washing of the wound with soap or water, application of human anti-rabies immunoglobulin and administration of vaccine according to type of exposure (Table 1.2) and official protocol approved by local authorities (Takayama, WHO, 2013).

Table 1.2. Type of contact, exposure and recommended post-exposure prophylaxis (WHO, 2013)

Category	Type of contact	Type of exposure	Recommended post-exposure prophylaxis
I	• Touching or feeding of animals • Licks on intact skin	None	None, if reliable case history is available
II	• Nibbling of uncover skin • Minor scratches or abrasions without bleeding	Minor	• Administer vaccine immediately • Stop treatment if the animal remains healthy throughout period of 10 days or is proved to be negative for rabies by a reliable laboratory using appropriate diagnostic techniques • Wound management
III	• Single or multiple transdermal bites or scratches, licks on broken skin • Contamination of mucous membrane with saliva (i.e. licks) • Suspect contact with bats	Severe	• Wound management • Rabies immunoglobulin • Anti-rabies vaccine

b. Control of rabies in dog population

The main measures advocated for control of rabies in dog population consist in dog population management and vaccination against rabies which must be supported by socio-economic and administrative determinants such as dog ownership, legislation, public awareness, surveillance and laboratory-based surveillance, intersectoral collaboration, regional and international collaboration and cooperation (WHO, 1987).

b.1. Dog population management

The dog population management program includes the movement restriction, habitat control and reduction of dog density through birth control and culling of stray and rabid dogs (Knobel et al., 2007). Jointly with the strict application of the legislation, the dog population management has been carried out since half of the 19th century in Europe where it has contributed significantly to the control of rabies before discovery of veterinary rabies vaccine in 1920 (Rupprecht et al., 2002).

The animal birth control (ABC) program of dog population includes surgical sterilization (castration, ovariohysterectomy), chemical sterilization (delivery of hormonal contraceptives) and confinement of dogs (FAO, 2014). The ABC program in combination with vaccination has been proposed in 1960 as way of reducing unsupervised dog numbers, dog density, population turnover and rabies incidence, leading to reduction of human bite injuries and creating a stable, immunized dog population. Although successful results were reported in a number of countries (e.g. India) thanks to ABC program, independent evaluation of impact and cost-effectiveness of this strategy is needed (WHO, 2005; Reece and Chawla, 2006; Knobel et al., 2007).

Culling of dogs is used alone or with vaccination (Kaplan et al., 1954; Larghi et al. 1988) based on assumption that rabies transmission is dependent on dog density and is likely to be maintained endemically in areas where dog density exceeds the threshold for persistence, considered to be about 5 dogs/km^2 (Brooks, 1990; Cleaveland and Dye, 1995; Kitala et al., 2002; Lembo et al., 2008). Culling or removal and destruction of dogs is considered as contrary to animal welfare and seems to be ineffective in controlling rabies (Morters ate al., 2015). Indeed, culling is negatively perceived in society and results in lack of community support in fight against rabies. In addition, due to fear of culling, owners may relocate dogs to an area where rabies is not currently prevalent or may seek a replacement dog from a rabies-prevalent area and reintroduce the disease (Lechénne et al., 2015).

b.2. Mass vaccination

The first effective veterinary vaccine against rabies was an attenuated live virus. It was developed in 1920 by Umeno and Doi (1921) in Japan. Then, various rabies vaccines were developed and used for the control or elimination of rabies in domestic and wild animals such as parenteral (injectable) modified live vaccine, parenteral inactivated vaccine, oral modified live vaccine and new generation of biotechnology-derived vaccines (WHO, 2013; Yang et al., 2013). Since the event of veterinary vaccine, the dog rabies was controlled or eliminated in several countries of Europe, North America, Latina America and some Asian countries through combination of mass vaccination and classical control measures. Japan was the first country to apply mass vaccination of dogs in 1921, whereas Hungary was the first country to have experienced the elimination of dog rabies in 1944 through massive vaccination implemented from 1939 to 1944 (WHO, 1966; Manninger, 1968; Knobel et al., 2007).

In addition, the routine vaccination of pet animals against rabies is still mandatory particularly in rabies endemic countries. Vaccination guidelines are recommended by vaccine manufactures and scientists such as the Vaccine Guideline Group (VGP) (Day et al., 2006). But each country may adjust the guideline to its epidemiologic situation.

Field and modelling studies have demonstrated that a vaccination coverage of at least 40%, called critical threshold, was required to prevent rabies transmission and large outbreaks (Coleman and Dye, 1996), whereas a coverage of at least 60%, called control threshold, was sufficient for the control or elimination of rabies either through mass vaccination campaigns using parenteral (sub-cutaneous injection) vaccination strategy (Steele and Tierkel, 1949; Cleaveland et al., 2003; Schneider et al., 2007) or combination of parenteral and oral vaccination strategies (Ben Youssef *et al*., 1998; Bishop, 2001). The oral vaccination may thus allow for improvement in dog vaccination coverage or for the targeting of high-risk, inaccessible segments of the population as vaccines are included within attractive bait for oral consumption. However, oral vaccines are more expensive. In addition, due to the high affinity between dogs and humans, the oral vaccines need to be efficacious as well as safe. Baits should be preferentially attractive to dogs and not to non-target species. To date , no universal bait has been identified, although many different types have been evaluated successfully (Knobel et al., 2007; Cliquet et al., 2018).

b.3. Socio-economic and administrative determinants of dog rabies control

The socio-economic and administrative determinants include: (i) the community engagement and public awareness which are essential for good dog-keeping-practices , high accessibility of dogs to vaccination and thereby increasing the vaccination coverage; (ii) surveillance and intersectoral collaboration which are important tools in rabies control, for assessment of impact of rabies control program and particularly for the effective and economic application of control measures in animals and post-exposure treatment in man, and (iii) regional and international technical collaboration that are essential in resource mobilization and coordination of control activities (canine rabies elimination in border areas and measures governing the import of dogs, cats and other animals able to spread rabies, etc.) (WHO, 1987; Léchenne et al., 2015).

Chapter 2. Factors of maintenance of rabies transmission in dogs in Kinshasa, Democratic Republic of the Congo

The overview provided evidence that rabies is still maintained in dog population in Kinshasa, although the incidence is underestimated. This research aimed at unravelling risk factors of rabies maintenance in dogs in urban settings and identifying risk zones. It refers to the first specific objective of this thesis. The study highlighted two major risk factors of rabies transmission in dogs, namely the poor dog-keeping practices and low vaccination coverage. It appears that those risk factors are influenced by socioeconomic status of households, thereby leading to different risk zones within urban settings. These are low, moderate and high risk zones of dog rabies transmission. Finally, the study proposed a tool for establishing a low scale risk map that can be used for local risk assessment and targeting high-priority risk zones for rabies control.

This chapter was adapted from:

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2.1. Abstract

Rabies kills every year 61 000 humans in the world, mainly in Asia and Africa. In Democratic Republic of the Congo (DRC), dog rabies is endemic. Despite mandatory vaccination of dogs since 1938, disease control remains ineffective. Accordingly, this research aimed at unraveling risk factors of rabies maintenance in dogs in urban settings and identifying risk zones by combining the dog density, dog roaming behaviour and vaccination coverage. The method used to estimate the three factors was a household survey conducted in 22 study sites of Mont-Ngafula, Ngaliema and Lemba *communes* in Kinshasa. In addition to household survey, (i) a subgroup of owned dogs (n=16) was tracked during 24 hours using GPS collars in order to characterize the dog roaming behaviour, (ii) the feral dog proportion was estimated by the street count method , and (iii) the serological evaluation of the immunization status of a subgroup of 132 reported vaccinated dogs. The survey included 6122 households. In total, 504 dog-owning households with 922 dogs were recorded, corresponding to 9% of all households. The mean age of dogs was 2.5 years and 60% of dogs belonged to local breeds. Dog density was estimated to 49 dogs/km^2. Between 2 to 100% (mean 60%) of owned dogs were intermittently or continuously free roaming in study sites. The mean distance covered by tracked dogs within 24 hours was 0.718 km (0.046-2.341 km) and each dog had a chance to come in contact with 30 free-roaming dogs. The proportion of feral dogs was less than 2%. The vaccination coverage was 53% (24-81% among study sites). The coverage significantly increased with age and was higher in pure and cross breed dogs. Associated costs and low age were reported as main reasons for not vaccinating. 73% of the 132 tested dogs displayed protective anti-rabies antibody titers (≥0.5 IU/ml) irrespective of time span since vaccination. By combining the dog density, the percentage of free-roaming dogs and the vaccination coverage, the risk map indicated a high risk of rabies transmission in 41% (9/22) of the study sites. Our study shows that the risk of rabies transmission varies locally in urban settings in Kinshasa. Dog-keeping practices and vaccination coverage correlate with the socioeconomic status of households and thereby influence the risk level of dog rabies transmission. The establishment of a low scale risk map provides a tool for local risk assessment and targeting areas and/or action aiming at rabies control.

2.2. Introduction

Rabies is a zoonotic disease responsible for an estimated 61 000 human deaths per year in the world, predominantly in Asia and Africa (WHO, 2013). A wide range of mammals are susceptible and can transmit rabies. The order of carnivora including domestic dogs (*Canis lupus*), raccoons (*Procyon lotor*), skunks (*Spilogale putorius*), foxes (*Vulpes vulpes*), jackals (*Canis aureus*) and the order of Chiroptera (bats) are considered as reservoirs (Sedganti and al., 1990). The dog is responsible for 98% of rabies cases in Africa and Asia (Knobel et al., 2005).

The first rabies outbreak in Africa was reported in Algeria in 1858 (Steel, 1975). In the Democratic Republic of Congo (DRC), the first dog rabies outbreak was reported in 1923 (Repetto, 1932). Then, from 1938 to 2017, the published and unpublished laboratory data revealed that close to 1400 dog rabies cases were confirmed across the country by the three national veterinary laboratories of Kisangani, Lubumbashi and Kinshasa (Courtois et al., 1964; Makumbu, 1977; Bula and Mafwala, 1988; Twabela et al., 2016). In Kinshasa, the capital of the DRC, 152 dog-related human rabies cases were reported from 2009 to 2017. Most of these victims were children under 15 years old (Muyila et al., 2014; OVCR, unpublished data). It is likely that these official rabies data are under-reported. Indeed, active surveillance studies illustrated that official reports underestimate the abundance of rabies cases in low-income countries such as in Tanzania, Ethiopia and Bhutan (Cleaveland et al. 2002; Hampson et al., 2008; Deresa et al., 2010; Tenzin et al., 2011; Jemberu et al., 2013). In the DRC, field evaluations have evidenced the poor performances of veterinary services. These are explained by inefficient surveillance system and limited diagnostic capacity of national veterinary laboratories (Niang and Denormandie, 2008; Diop et al., 2012; Ministère de la Pêche et de l'Elevage, 2017).

Nearly one century after the first reported rabies outbreak (Repetto, 1932), dog rabies is still a public health threat in the DRC. Given that the World Health Organization (WHO), the World Organization for Animal Health (OIE) and the Food and Agriculture Organization (FAO) have set a global target of zero human deaths from dog-transmitted rabies by 2030 (Global Alliance for Rabies Control, 2015; Wallace et al., 2017; Fahrion et al., 2017), the challenge for DRC remains considerable. Despite mandatory rabies vaccination of dogs since 1938 in DRC (Royal Decree of 01 April 1938) disease control remains ineffective. It is therefore important to investigate the reasons for the maintenance of rabies in dog populations

and identify regions presenting the highest risk of rabies transmission. Risk factors such as dog density, poor dog management leading to free roaming, low vaccination coverage and wide biodiversity increasing the number of the rabies virus reservoirs have been identified in other countries such as in Zimbabwe, Tanzania (Foggin, 1988; Brooks, 1990; Cleaveland and Dye, 1995; Aréchiga et al.,2014), but no data are so far available for DRC.

Accordingly, the aims of this study were (i) to investigate the risk factors of rabies transmission between dogs in Kinshasa and (ii) to establish a risk map of rabies transmission by considering these risk factors. Risk factor assessment included the characterization of the dog population and its management as well as the evaluation of dogs' vaccination coverage against rabies in Kinshasa.

2.3. Materials and methods

2.3.1. Study area

The study area was the capital of DRC, Kinshasa. This megalopolis is divided in 24 *communes*, further subdivided in *quartiers* including plots with one or more households (Decree N°08/016, 07 October 2008). The study was conducted from January 2017 to March 2018 in three *communes* where most dog rabies cases had been reported by the "Office de Vaccination et Contrôle de la Rage (OVCR)" in Kinshasa between 2003 and 2017 (unpublished data), ie Mont-Ngafula, Ngaliema and Lemba. In these *communes*, 22 *quartiers* were selected as primary sample units (study sites).

2.3.2. Characterization and management of the dog population

a. Dog density and population structure

A household questionnaire survey was conducted in the 22 study sites by selecting at least 20 dog-owning households per site. In each study site, the investigators walked in the streets, visited plots and contacted each household until 20 households with at least one dog were reached. All households with no dog were also recorded. This purposive sampling was done instead of random or systematic sampling because household lists and numbers for each study sites were not available. We assumed that households were sufficiently homogenous for important selection biases not occurring.

A questionnaire was used to collect data including: (i) the number of households on the plot, (ii) the number of dog-owning households, (iii) the number of households with no

dogs, (iv) the number of people living in dog-owning households, (v) the number of people living in households with no dogs, (vi) the number of dogs owned per household and (vii) the individual description of owned dogs (gender, age and breed). The identified dogs were classified according to sex (male, female), age (puppies: less than 3 months, juveniles: from 3 to 12 months and adults: more than 12 months old) and breed (local breeds, crossed breeds, pure breeds). Data were expressed as relative frequencies.

The dog density was estimated from the ratio between the projected human density and the estimated Human to Dog Ratio (HDR). The projected human density data was obtained from the civil administration. The HDR is one of the best indicators of dog population abundance (WHO, 1987; Oboegbulem and Nwakonobi, 1989). It was calculated from the ratio between the total number of people recorded in visited households with or without dog and the total number of dogs recorded in visited dog owning-households. Ownerless dogs (see below) were excluded from the calculation of dog density.

b. Dog management

In order to estimate the proportion of restrained and free roaming owned dogs, the household questionnaire also addressed (i) the level of dog confinement (fully tied or caged, intermittently tied or caged, free roaming), (ii) the type of plot (plot with or without fence/wall or any physical barrier that restrained dog's movement), and (iii) the dog feeding (provided by the owner or ensured by the dog itself during roaming). In addition, the reasons for dog abandonment were addressed in open questions.

An owned dog was considered as restrained if fencing, tying or caging completely prevented its roaming behaviour. All intermittently or non-restrained dogs were considered as potentially free-roaming. Results regarding restrained and roaming dogs, as well as reasons for dog abandonment were also expressed as relative frequencies.

In order to evaluate dogs' roaming behaviour, 16 free-roaming dogs (8 males and 8 females) owned by members of the academic staff of the University of Kinshasa (UNIKIN) and inhabiting the University campus were tracked during 24 hours using GPS collars. The majority of these dogs (15/16) were adults (≥ 12 months). The GPS I-GOTU GT-600 (I-gotU company) was programmed to take a GPS location each minute. The maximum distance covered by each dog was calculated based on GPS coordinates of the household and the most distant record using the formula available at http://www.ipnas.org/garnir/donneesGPS. In

addition, the direct or indirect contact rate of tracked dogs with other free-roaming dogs was iteratively estimated in four steps by using the Quantum GIS software (http://www. qgis.org): (i) generation of a buffer zone which refers to the potential area covered by a tracked dog. The radius of the buffer zone corresponded to the maximum distance covered by each tracked dog, (ii) calculation of the area of the administrative (*quartier*) unit that was covered by the buffer zone and that we call "intersection area", (iii) estimation of the number of potentially free-roaming dogs per intersection area by considering the calculated dog density and the percentage of potentially free-roaming dogs in each respective *quartier*, and (iv) estimation of the contact rate with free-roaming dogs within the buffer zone by summarizing the number of dogs in intersection areas.

The percentage of feral dogs was assessed in two study sites (Mitendi and Mongala) of the *communes* Monga-Ngafula and Ngaliema by the street count method, which is a modification of the sight-resight method (WHO, 1987). A total of 185 (Mitendi) and 110 (Mongala) owned dogs were identified with a yellow nylon rope used as collar. The following day, dog counters walked once in the morning (8 am) and once in the evening (6 pm) through the study sites and recorded identified and non-identified free-roaming dogs

2.3.3. Rabies vaccination

a. Vaccination coverage

The household survey also assessed the vaccination status of owned dogs by considering owner's report (history of vaccination and time point of last vaccination) or the vaccination certificate (if available). Reasons for not vaccinating dogs were addressed by semi-structured questions. The vaccination coverage was estimated for each study site from the ratio between the numbers of reported vaccinated dogs (independently of time since vaccination) and the number of identified dogs, including puppies of less than three months.

The vaccination status of the dogs (binary variable: vaccinated or not) was analysed using a cluster robust multivariable logistic regression in STATA software 11.0 (Stata Corp., college Station, Texas). Categorical explanatory variables were the sex of the animals (male, female), their age categories (puppies, juveniles, adults), their breed (local, crossed, pure breeds) and management (free, non-roaming). The robust model, which is more conservative, accounts for a possible design effects (DEFT) caused by the 22 study sites considered as clusters or primary sampling units. The relevance of the cluster robust model was evaluated

by calculating and evaluating DEFT for each explanatory variable (Kreuter and Valliant, 2007).

The owner's reasons for not vaccinating dogs were aggregated and results were expressed as relative frequencies.

b. Serological evaluation of the immunization status of vaccinated dogs

Further to oral consent of the owners, 132 supposed vaccinated dogs aged between six months and fourteen years of Mont-Ngafula, Ngaliema and Lemba *communes* underwent venous blood collection. Serum was harvested after centrifugation and stored at -20°C. Anti-rabies antibody detection was performed by Sciensano National Reference Laboratory of Rabies in Belgium by use of Rapid Fluorescent Focus Inhibition Test (RFFIT), one of the WHO and OIE reference methods (Meslin et al., 1973; OIE, 2014).

Antibody titers were expressed in International Units per milliliter (IU/ml) and 0.5 IU/ml of anti-rabies antibody was considered as the minimum protective titer (WHO recommendations, 1992). Results were analyzed as regards of protective antirabies antibody titer (< and ≥0.5 IU/ml) and the time span since last vaccination (≤1 year, 1-2 years, 2-3 years, >3 years). Using the STATA software, a logistic regression model was used to explore if the percentage of vaccinated dogs with protective titer differed by the time span since last vaccination.

2.3.4. Risk map establishment

The risk of rabies transmission among dog populations was assessed by combining results of vaccination coverage, roaming behaviour and dog density in order to establish a risk of level 1 (low), 2 (medium) or 3 (high) for each study site. A weighting score was given to the different levels of the risk factors, namely vaccination coverage, roaming behaviour and dog density. Thresholds were used in order to establish categories of vaccination coverage: ≥ 60%, 40-60% and <40% (Coleman and Dye, 1996; Hampson et al., 2009); percentage of free roaming dogs: ≤25%, >25-50%, >50-75%, >75-100% and dog density: < 5 and > 5 dogs/km^2 (Table 2.1).

Table 2.1. Risk factor categories used for establishment of rabies transmission risks among dog populations

Risk factors	Weight (%) of each risk factor (w)	Threshold	Score of each threshold (s)	Weighted score of each threshold (w*s)
Vaccination coverage (%)	60	≥ 60[a]	1	0.6
		40-60	2	1.2
		< 40[b]	3	1.8
Percentage of free roaming dogs	30	≤ 25	1	0.3
		> 25-50	2	0.6
		> 50-75	3	0.9
		> 75-100	4	1.2
Dog density (dogs/km^2)	10	< 5	1	0.1
		≥ 5[c]	2	0.2

Notes: The risk per study site was the sum of three weighted scores by combination of three risk factors, which could be equal to 1 (low risk), 2 (medium risk) or 3 (high risk).

[a] Empirical rabies control threshold (Cleveland et al., 2003; WHO, 2013).

[b] Under the critical threshold (Coleman and Dye, 1996; Hampson et al., 2009)

[c]Dog density threshold for rabies maintenance in dogs in endemic regions of Africa (Foggin, 1988; Brooks, 1990, Cleaveland & Dye, 1995; Kitala et al. 2002, Lembo et al. 2008)

2.4. Results

2.4.1. Characterization and management of the dog population

a. Dog density and population structure

The household survey included 6122 households located in 2914 plots. In total, 504 dog-owning households with 922 dogs were recorded, corresponding to 9% (95% CI: 8- 10%) of all households. In most visited dog-owning households, the dog owner accepted to participate in the study. The average number of dogs per dog-owning household was estimated to 1.8 dogs (95% CI: 1.7-1.9). The Human to Dog Ratio equaled 53 (95% CI: 49-57) and the dog density was estimated to 49 dogs/km^2 (95% CI: 40-58), with a range of 22-90 dogs/km^2 in study sites.

Fifty eight percent of recorded dogs (535/922) were males. Furthermore, close to 60% of dogs were adults (≥ 12 months of age), whereas puppies (≤ 3 months of age) represented 15% of the population. The mean age of dogs was 2.5 years (95% CI: 2.2-2.8) and the majority (60%) of dogs belonged to local breeds (Table 2.3).

b. Dog management

Between 5 and 100% (mean 56%) of plots were insufficiently fenced and did not prevent dogs' roaming as illustrated in Figure S2.1. Regarding intermittently or continuously free roaming owned dogs, their percentage ranged from 2 to 100% across study sites (mean 60%, Fig. 3.1a). The study also showed that 0% to 94% (mean 46%) of dogs were either partially fed or not fed by their owners across study sites.

The analysis of GPS data showed that the 16 tracked dogs covered maximum distances ranging from 0.046 to 2.34 km (mean 0.72 km). Maximum distances (> 2 km) were covered by males and highest roaming activities were recorded in the morning (before 8 am) and in the evening (after 6 pm). The estimated contact rate with other dogs equaled 30 (95% CI:23- 37) (Fig. 2.1).

Among 201 free-roaming dogs recorded by street count in two study sites, three were deemed to be ownerless (1/131 and 2/70). The average proportion of feral dogs was less than 2%. Regarding owners' attitude toward dog's abandonment, only 8% (40 among 504 owners) appeared to consider this option (Table S1.1).

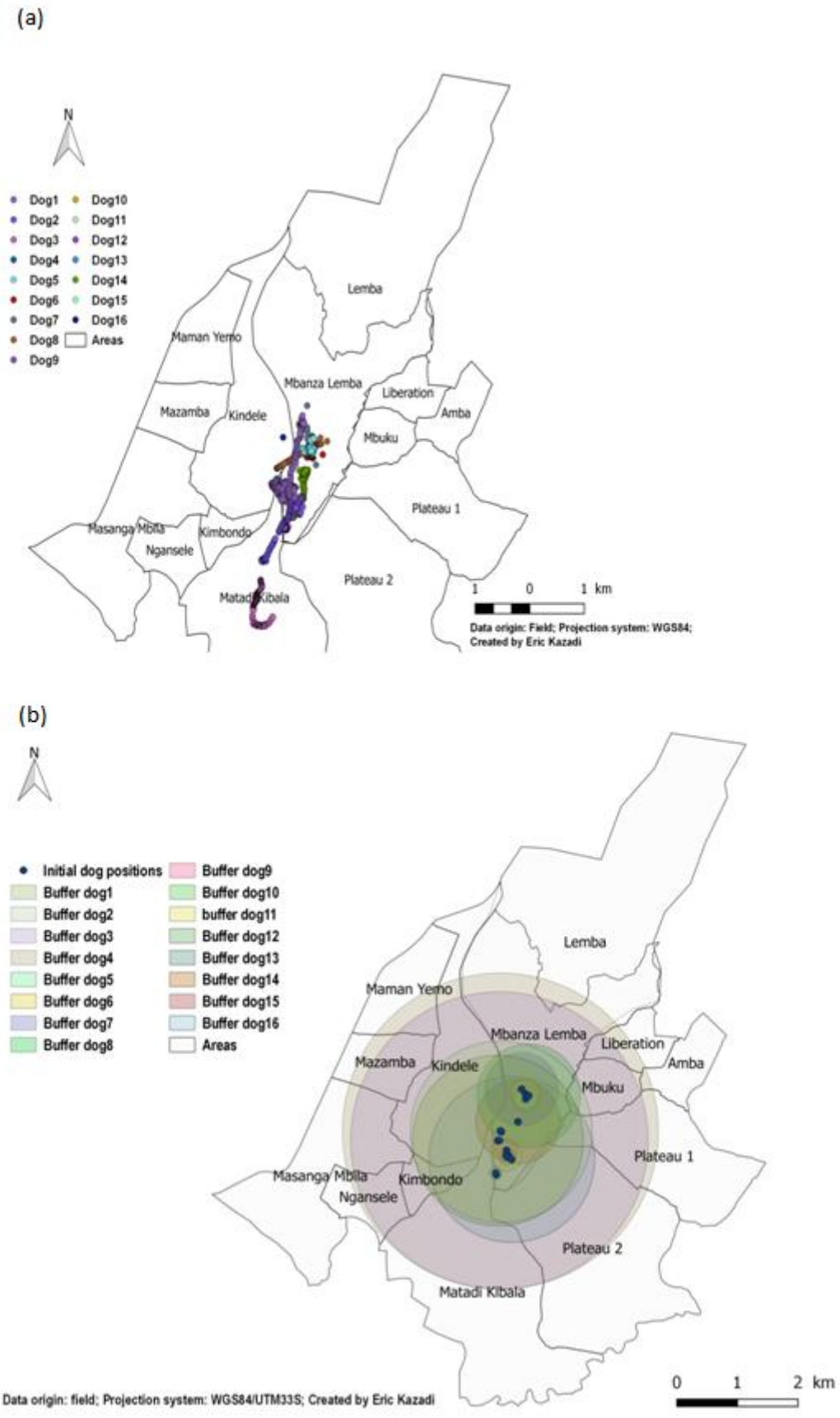

Figure 2.1. Maps showing the real movement of tracked dogs (n=16) within a 24 h period (a) and the buffer zones generated from the maximal distance covered by each dog based (b).

2.4.2. Rabies vaccination

a. Vaccination coverage

Fifty three percent (479/922) of dogs were reported to be vaccinated against rabies (the vaccination certificate was available for 89% of these dogs) and no differences with regard to sex and roaming behaviour were found. Vaccination coverage increased with age and was higher in pure and cross breed dogs (Table 2.3). Vaccination coverage ranged from 24% to 81% among study sites and was below the critical threshold of 40% in 8 of the 22 study sites (Fig. 2.3b). Associated costs and low age were reported as main reasons for not vaccinating dogs (Table 2.2).

Table 2.2. Owner's stated reasons for not vaccinating dogs against rabies

Reasons of non vaccination	Number of answers	Percentage
The lack of money or the high cost of the rabies vaccination	194	46%
The dog is too young (≤3 months or <1 year)	152	36%
The dog is not aggressive	68	16%
The lack of knowledge of the disease and the importance of vaccination	62	15%
The ignorance of the location of veterinary services	61	14%
The dog is completely restrained (no roaming)	59	14%
The negligence	32	8%
The vaccination side effects (loss of agressivity, death), the vaccinator's credibility	26	6%
The rabies vaccination is the Government's responsibility	14	3%
The bitch was vaccinated	14	3%
No data	9	2%
Total of answers	**424**	

b. Serological evaluation of the immunization status of vaccinated dogs

Seventy three percent of a subgroup of 132 reported vaccinated dogs displayed protective anti-rabies antibody titers (≥0.5 IU/ml). The percentage of protected dogs tended to decrease in function of time span since last vaccination from 81 to 63%, but this was not statistically significant (p=0.4, Fig. 2.2). Regarding dogs' age at the time of the first or last vaccination, dogs were vaccinated at about 12 months of age (median), ranging from 3 to 115 months. Independently of dogs' age at vaccination, the median time span since last vaccination was 18 months. This period varied from 3 to 90 months (data not shown).

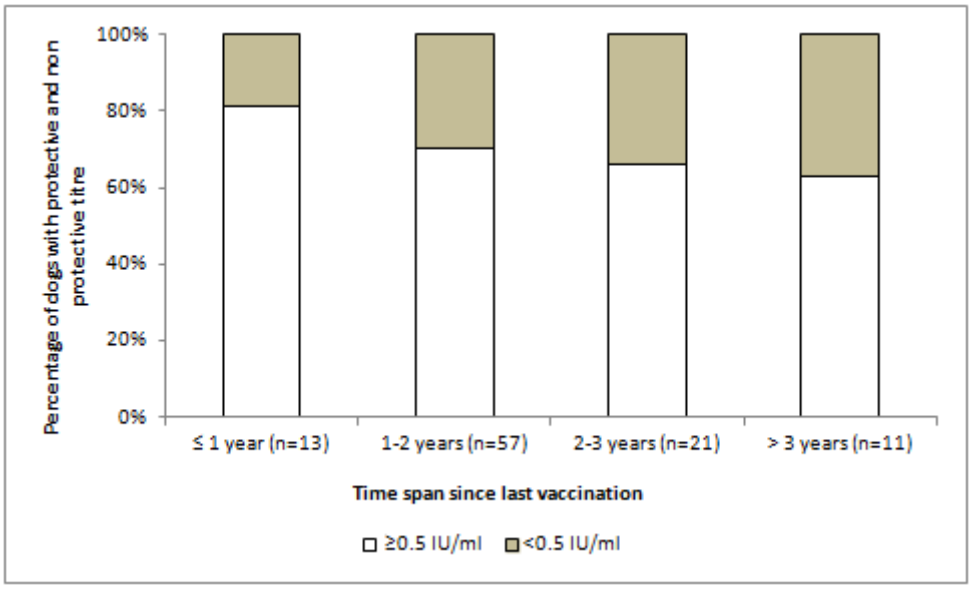

Figure 2.2. Distribution of serological status of reported vaccinated dogs in function of time since last vaccination: a: ≤1 year; b: 1-2 years; c: 2-3 years; d: >3 years. The proportion of dogs with protective titre (>0.5 IU/ml) does not differ between groups (p=0.4, logistic regression model).

2.4.3. Establishment of a risk map

The combination of vaccination coverage, roaming behaviour and dog density revealed that the risk level of rabies transmission among dog populations was 1 (low), 2 (medium) and 3 (high), respectively, in 27% (6/22), 32% (7/22) and 41% (9/22) of the study sites (Fig. 2.3c).

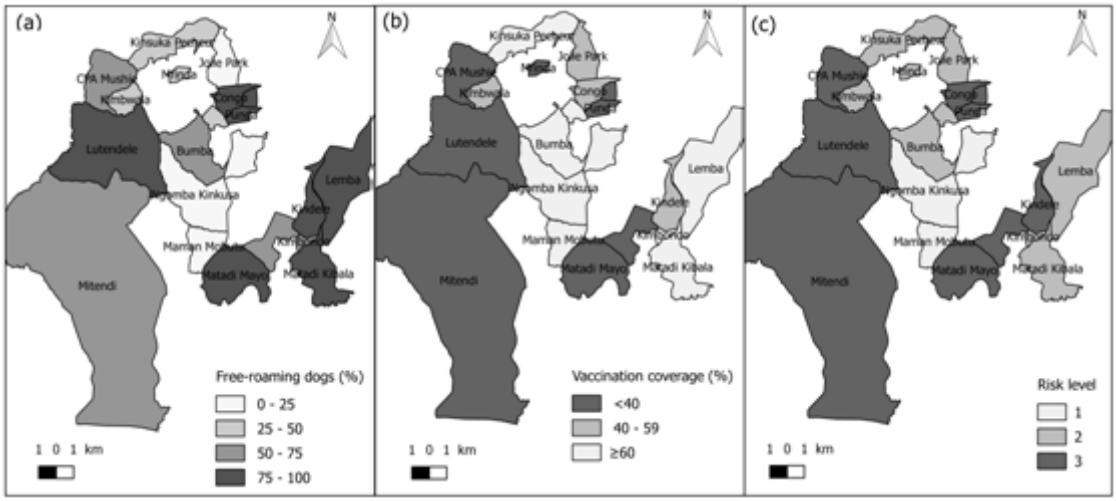

Figure 2.3. Selected study sites (*quartiers*) in Mont-Ngafula, Ngaliema and Lemba *communes*. (a) Estimated percentage of owned dogs which are potentially free to roam. (b) Estimated vaccination coverage. (c) Qualitative assessment of the risk of dog rabies transmission in study sites based on dog density, dog vaccination coverage and percentage of free-roaming dogs as the main risk factors.

Table 2.3. Characteristics of reported rabies-vaccinated dogs among 922 owned dogs, using a cluster robust logistic regression and multivariable model

Factors	Number of dogs	Proportion (%)	Number of vaccinated dogs	OR(95%CI)	P value	Prediction of vaccination coverage (95% CI)
Sex						
Male[a]	525	57	305			58 (54-62)
Female	397	43	305	1.5 (0.9-2.3)	0.113	52 (47-57)
Age categories						
Adults (>12 mo)[a]	504	56	380			74 (70-78)
Juveniles (3-12 mo)	280	30	122	13 (2.3-76.7)	**0.001**	44 (38-50)
Puppies (≤ 3 mo)	129	14	8	61 (9-413)	**0.001**	6 (3-11)
Dog management						
Non-roaming dogs[a]	390	42	273			70 (65-74)
Free- roaming dogs	532	58	237	1.6 (0.8-2.9)	0.111	45 (40-49)
Dog breeds						
Pure breeds[a]	106	12	101			95 (89-98)
Crossed breeds	271	29	174	3 (2.2-5.2)	**0.001**	64 (58-70)
Local breeds	545	59	235	23 (8.6-62.7)	**0.001**	43 (39- 47**)**

Abbreviation: mo months

[a] Reference variable represents the highest vaccinated category

2.5. Discussion

The present study aimed at unravelling risk factors of rabies transmission maintenance in dogs and identifying risk zones risk zones by combining the dog density, dog roaming behaviour and dog vaccination coverage.

The method used to estimate the three factors was the household questionnaire survey for which the accuracy of estimates (vaccination coverage, dog density) was not proven to be significantly different of those from census method, which is considered as the gold standard method (Cleaveland et al., 2003; Minyoo et al., 2015). In particular for dog density calculation, our method aimed at increasing the accuracy by considering the number of people living in households with no dogs in the calculating of HDR given the poor accuracy of available human population data and the lack of dog population data. Indeed, the last population census in the DRC was conducted in 1984 and the rural-urban drift is increasing (Flouriot, 2013), thereby justifying an update. Although the registration of dogs at the veterinary services is mandatory since 1918 (Royal Decree of 22 January 1918) in the DRC, the law is not respected by owners.

Possible biases include response, classification and selection biases. The response bias was low since people were found in most households and very few refused to answer the questionnaire. Mis-classification could occur as people might fear to declare they owned dogs that were not vaccinated. Finally, a selection bias could have occurred because of the purposive sampling strategy.

Presently, the inclusion of the dog density among risk factors of rabies transmission in dog populations is debatable. On one hand, several field and modelling studies demonstrated a density-dependency of rabies transmission in Africa, where the disease persists in dog populations with a density > 5 dogs/ km^2 and only sporadically appears under this threshold (Foggin, 1988; Brooks, 1990, Cleaveland and Dye; 1995; Kitala et al. 2002). On the other hand, the study of Morters et al. (2013) found no conclusive evidence that support the relationship between dog density and rabies transmission. In our study, dog density equaled 49 dogs/km^2, which is almost ten times more than the above threshold density (5 dogs/km^2). Densities varied depending on *quartiers* (min 22 – max 90 dogs/km^2).

The second risk factor was the poor dog management because more than 50% of owned dogs were free roaming in 60% of the study sites (Fig. 2.3a). The main reasons for

roaming were the absence of a physical barrier that permanently prevented dogs from roaming and the owners who voluntarily allow dogs to roam in search for food in public dumps and open markets.

The total roaming restriction of all dogs should be the first measure of rabies control at the community level as applied in parts of Europe before implementation of vaccination programs (Wallace et al., 2017). In Kinshasa, the total restriction of dogs is not feasible due to above mentioned reasons of abundance of free-roaming dogs. However, it can be considered that most free-roaming dogs might be easily captured and punctually caged or tied for vaccination as they have owners. Indeed, apparently feral dogs accounted for less than 2% of the free-roaming dog population in the two study sites. The term "apparently feral dog" was used instead of "feral dog" because the street-count method used to estimate the percentage of ownerless dogs could not exclude the presence of owned and feral dogs from neighbouring areas. The estimated percentage of ownerless dogs was low (≤ 2%) and in line with estimates of 0–11% ownerless dogs in Zimbabwe, Tanzania and Chad (Butler and Binghame, 2000; Cleaveland, 2014). Considering the mean *quartier* size (6,1 km^2), the mean roaming distance of dogs (0.72 km) and the roamed surface (1.6 km^2), it can be hypothesized that roaming dogs, whether they are owned or not, mainly roam within one or two *quartiers*. Such information is important for vaccination campaigns because it suggests that high vaccination coverage could be achieved very locally. Given the reduced (n=16) number of dogs whose roaming behaviour was assessed by GPS tracking, further investigations implying a larger number of dogs that are housed in different study sites would be useful.

Vaccination against rabies remains the key component of rabies control as shown by the strong correlation between high vaccination coverage and low rabies incidence demonstrated in several studies. Indeed, the empirical vaccination coverage of ≥ 60% has led to a significant reduction of rabies outbreaks (Korns and Zeissig, 1948; Cleaveland et al., 2003; Hampson et al., 2009; Morters et al. 2013; Global Alliance for Rabies Control, 2015). In contrast, rabies outbreaks occur when the immunization coverage falls under the critical threshold of 40% (Coleman and Dye, 1996; Hampson et al., 2009). Several methods can be used to estimate the vaccination coverage (Minyoo et al., 2015). In the present study, the household questionnaire survey recorded vaccinated dogs regardless of the time span since last vaccination. Among 132 blood-sampled dogs, 73% showed a protective antirabies antibody titre (≥0.5 IU/ml) and the impact of time span since last vaccination was not demonstrated (Fig. 2.2). This may be due to the small sample size. A decreased titer was

observed as the time since vaccination increased and the recommendation for annual vaccination in dogs (Arrêté N°SC/151/BGV/MIN/AGRI & DR/SMI/2016) is still valid. These results further suggest that a proportion of vaccinated dog populations with a poor turnover would be protected against rabies for more than one year. Furthermore, it can be speculated that reported vaccinated dogs without protective antirabies antibody titres (27 % of dogs with <0.5 IU/ml) had nevertheless been immunized against rabies and that they would display a rapid memory immune response upon exposure. On the other hand, a lack of quality (potency) of the vaccine due to an inadequate cold chain during vaccine storage or non-responding dogs could also account for absence of protection (Day et al., 2016).

By considering the vaccination history of all owned dogs through the household questionnaire, the overall vaccination coverage equaled 53% and was above the critical coverage level of 40% (Coleman and Dye, 1996; Hampson et al., 2009). However, the coverage significantly differed between study sites and ranged from 24 to 81%. In addition, coverage in 36% (8/22) of study sites was below the critical immunization of 40%, which is propitious for rabies outbreaks (Hampson et al., 2009). It is important to emphasize that the low coverage (<40%) was estimated particularly in areas with low proportions of restricted dogs (Fig 2b). The variability of coverage between study sites is likely to be linked to the differences of the socio-economic situation of their inhabitants. Despite mandatory vaccination of dogs against rabies in DRC (Royal Decree of 01 April 1938), vaccination is not fully applied in the field and must be afforded by the dog owners. Indeed, the current cost (20 USD) for rabies vaccination appears as the first reason of non-vaccination for 46% of the interviewed dog owners (Table 2.2). Given that in DRC 70% of people live under the poverty threshold (Moummi, 2010), it might be expected that low-income households own non-vaccinated dogs. Another consequence of poverty is a poor dog management: local and crossed breeds are less expensive (Kazadi et al., 2017) and are allowed to roam freely, whereas pure breeds predominantly live in fenced plots.

The dogs' age was another factor limiting vaccination. Most of the dogs under one year of age, and mainly puppies (≤ 3 months of age), were often unvaccinated. The WHO recommends the inclusion of puppies of less than three months of age in the rabies vaccination programs (WHO, 2013). Indeed, puppies are susceptible sub-populations and published laboratory data show that 4 to 17% of confirmed rabies cases are puppies under three months (Perry, 1993; Widdowson et al., 2002; Reta et al., 2014; Morters et al., 2015). However, many owners, veterinarians and veterinary assistants consider that puppies are too

young for vaccination. As a consequence, 94% (121 of 129) puppies, presenting 14% of the dog population were not vaccinated (Table 2.3).

Finally, the combination of the three main risk factors in form of a risk map reflected the likelihood of rabies transmission. This risk was found to be high and medium respectively in 41% (9/22) and 32% (7/22) of study sites (Fig 2.3c). In addition, it is likely that *quartiers* that are close to high risk sites should be cautiously regarded as high risk sites. This key result correlates closely with the rabies epidemiological context (unpublished laboratory dog rabies data). The high risk level of rabies transmission was associated to poor dog-keeping practices and to low vaccination coverage. Both factors were tightly linked to the socioeconomic status of dog-owning households. Indeed, some dogs were not exclusively feed by the owners and were therefore allowed to roam freely. Furthermore, the cost of vaccination (ie 20 USD in DRC) is not affordable to most of owners in impoverished suburbs (Kazadi et., 2017). An association of increased risk for canine rabies and areas of low socioeconomic status has also been shown in Mexico and Bolivia based on positive rabies samples from different urban settings (Eng et al., 1993; Widdowson et al., 2002). In China, the low vaccination coverage and the growth of uncontrolled dog populations as a consequence of socio-economic changes were the main causes of rabies re-emergence in poor communities (Yin et al., 2013). Based on these evidences, the combination of the three main risk factors in form of a risk map provides a tool for the field assessment of rabies risk in urban settings. It should be added that in peri-urban and rural settings, the role of wild animals in the maintenance of rabies in dog needs further investigations.

2.6. Conclusion

Our study shows that the risk of rabies transmission varies locally in urban settings in Kinshasa. Dog-keeping practices and vaccination coverage correlate with the socioeconomic status of households and thereby influence the risk level of dog rabies transmission. The establishment of a low scale risk map at the level of *quartiers* and by considering vaccination coverage, roaming behaviour and dog density provides a tool for local risk assessment and might be useful for targeting areas and/or action aiming at rabies control.

Chapter 3. Evaluation of dog vaccination schemes against rabies in Kinshasa, Democratic Republic of the Congo

Based on rabies transmission risk zones set up in Chapter 2, this study aimed at proposing a risk-based vaccination strategy for rabies control in dog population. It corresponds to the second specific objective of this thesis. Four settings were targeted: two settings with low risk and low roaming dog population and two others with high risk and high roaming population. This study evidenced the link between the dog-keeping practices and dog turnover rate. Dog turnover was high in settings with poor dog-keeping practices (high roaming dog population) and high risk of rabies transmission, leading to rapid removal of vaccinated dogs, increase of susceptible dogs made up mainly of dogs less than 12 months of age , and conversely, to rapid decrease of proportion of vaccinated animals in the population. On the other hand, the systematic vaccination of puppies at weaning (3 months of age) results in a sufficient proportion of immune animals in high risk and high turnover rate settings. Assuming that a single vaccination at 3 months protects the animal for 3 years at least, the implementation of systematic vaccination of puppies at weaning seems to be efficient and more appropriate rabies vaccination strategy for rabies control in resource-poor and endemic countries, despite high turnover rates.

Article in preparation

3.1. Abstract

Dog-mediated human rabies is still endemic in Democratic Republic of the Congo (DRC), despite the fact that the disease can be controlled through mass dog vaccination campaigns that are not regularly organized in Kinshasa. No strategy is properly applied probably either because the State is unable to allocate resources for rabies control or due to inability of dog owner to afford vaccination costs. Given the existence of different risk zones countrywide, the efficiency of vaccination strategy may be improved by targeting high-risk zones. Therefore, the present study aimed at proposing a risk-based vaccination scheme by considering (i) the canine population dynamics with different risk profiles for rabies transmission and (i) the efficacy and duration of a serologically-detectable immunity in response to vaccination and revaccination tested under field conditions. The capital of DRC, Kinshasa, was chosen as study site for this rabies vaccination outcome model. The population dynamics was carried out in two low roaming populations (<25% of dogs are roaming) and in two high roaming zones (>75% of dogs are free roaming). Results evidenced the link between the dog-keeping practices and the turnover rate. It was twice as high in dog roaming settings (36%) as that in low-roaming zones (17%). Similar differences were found for birth rates (45% versus 32%) and dog removal rates (40% versus 9%). Irrespective of roaming level, dog populations were young: 75% of dogs were less than 3 years old. The vaccine was equally effective (p=0.24) in puppies (2-3 months: 96%), juvenile (3-12 months: 97%) and adult dogs (>12 months: 100%). The assessment of vaccine efficacy in terms of serological response to primo-vaccination showed that the vaccine was effective in 93% (11/12) of puppies that had no prevaccinal protective titers (≥0.5 IU/ml). The memory or anamnestic response was strong and rapid within 5-8 days upon the booster vaccination, in 96% (45/47) of dogs reported vaccinated for 1 to 7.5 years. This suggests that the duration of immunity provided by the rabies vaccine is lasting more than 3 years. Thus, given the short life expectancy (≤3 years) of 75% of surveyed dogs, it was hypothesized that vaccine may provide a lifetime protection against rabies for the majority of the investigated dog population. Considering the setting-specific turnover rate and the critical threshold of 40% under which rabies outbreaks occur, an initial proportion of 80% of immune dogs drops below 40%, respectively after 27 months in low risk and low dog turnover rate (17%) and 18 months in high risk and high turnover rate (36%). In contrast, when dogs are systematically vaccinated at 3 months of age, the proportion of immune dogs is maintained even in high risk and high turnover rate, thereby confirming the epidemiological needs for inclusion of puppies in vaccination program.

Annual vaccination campaigns, if implemented with a sufficient coverage, result in severely varying proportions of vaccinated animals in a year (high after vaccination and low just before). Theoretically, the implementation of systematic vaccination of puppies at weaning seems to be efficient and more appropriate rabies vaccination strategy for rabies control in resource-poor and endemic countries, despite high turnover rates.

3.2. Introduction

Rabies is an acute meningoencephalitis due to a *lyssavirus*. The disease is fatal in humans in absence of early treatment. Every year, rabies is responsible for more than 60,000 deaths across the world mainly in Asia and Africa (WHO, 2013). In Africa, close to 98% of human rabies cases are due to dog bites (Knobel and al., 2005). Dogs are considered as the main vector of the disease. In the Democratic Republic of Congo (DRC), dog rabies is endemic (Courtois et al., 1964; Makumbu, 1977; Bula and Mafwala, 1988; Muyila et al., 2014, Twabela et al., 2016). The disease burden is underestimated due to inefficient surveillance systems and limited diagnostic capacities of national veterinary laboratories (Niang and Denormandie, 2008; Diop et al., 2012; Ministère de la Pêche et de l'Elevage, 2017).

The elimination of dog-mediated human rabies is feasible through mass vaccination of dogs as successfully demonstrated in Europe, America and the Caribbean Islands (WHO, 2013; Taylor and Nel, 2015). Empirical and modelling studies show that an annual vaccination coverage of at least 60% of the dogs leads to rabies control (Cleveland et al., 2003; Hampson et al., 2009; WHO, 2013). Annual mass vaccination of dogs aims at maintaining the proportion of immune dogs high. Indeed, the vaccination coverage of at least 60% is considered as appropriate for disease control, whereas a herd immunity below the critical threshold of 40% strongly raises the probability of rabies outbreaks (Coleman and Dye, 1996; Hampson et al.,2009). In Africa, the proportion of immune dogs declines quickly because of high dog turnover rates (30%) and exclusion of puppies (≤ 3 months) from vaccination programs (Coleman and Dye, 1996, Hampson et al., 2009; Morters et al., 2015). Another reason for annual mass vaccination is that some countries such as DRC requireannual vaccination boosters (Arrêté N^{o}SC/151/BGV/MIN/AGRI & DR/SMI/2016; Day et al., 2016).

A review on dog rabies vaccination coverage in Africa showed that the coverage is close to 70% following a free of charge mass vaccination, but can easily fall to 18% if dog owners have to pay for the cost (Jibat et al., 2015). Regular free of charge mass dog

vaccination campaigns are not implemented in most of Sub-Saharan countries (Hotez and Kamath, 2009). Owners' compliance towards mandatory vaccination strongly depends on their socio-economic situation and the subsequent ability to afford vaccination costs. It further appears that the dog management is also influenced by socio-economic factors, thereby leading to different risk zones within countries and within towns (Kazadi et al., 2020). Accordingly, the efficiency and cost-effectiveness of rabies control could be improved by considering the canine population dynamic of different risk zones. In this context, it would further be interesting to question the benefit provided by annual revaccination.

The aim of this study was to propose a risk-based vaccination scheme or model by considering (i) canine populations with different risk profiles for rabies transmission and (ii) the efficacy and duration of a serologically-detectable immunity in response to vaccination and revaccination tested under field conditions. The capital of DRC, Kinshasa, was chosen as study site for this rabies vaccination outcome model.

3.3. Material and methods

3.3.1. Study design

Population structure and dynamics in dogs was assessed in two low and two high risk rabies transmission areas (Kazadi et al, 2020). Low transmission risk was linked to dog populations with low roaming activity (<25% of dogs are roaming) and high rabies vaccination coverage (≥60%), whereas high transmission risk was associated with high roaming activity (>75% of dogs are free roaming) and low vaccination coverage (<40%).

The efficacy of a serologically-detectable immunity was assessed in response to primo-vaccination in dogs belonging to different age groups (puppies, juvenile dogs, adult dogs) whereas the duration of immunity was evaluated serologically by revaccination in previously vaccinated dogs and by considering time span since vaccination and revaccination.

These data as well as initial immunity levels were used to implement a rabies vaccination outcome model allowing prediction of the impact of vaccination strategies on canine population immunity.

3.3.2. Study area

The study area was Kinshasa, the capital of the DRC, which is administratively subdivided in 24 *communes* further subdivided in *quartiers* (Decree N°08/016, 07 October 2008). Selected *communes* and *quartiers* are detailed in Figure 3.1 and Table 3.1. Areas of low transmission risk were Righini and Ngomba Kinkussa, whereas Livulu and Mongala were considered as *quartier* with a high risk of transmission (Kazadi et al., 2020). Serological assessment of rabies immunity was assessed in different *communes* based on dog owner compliance.

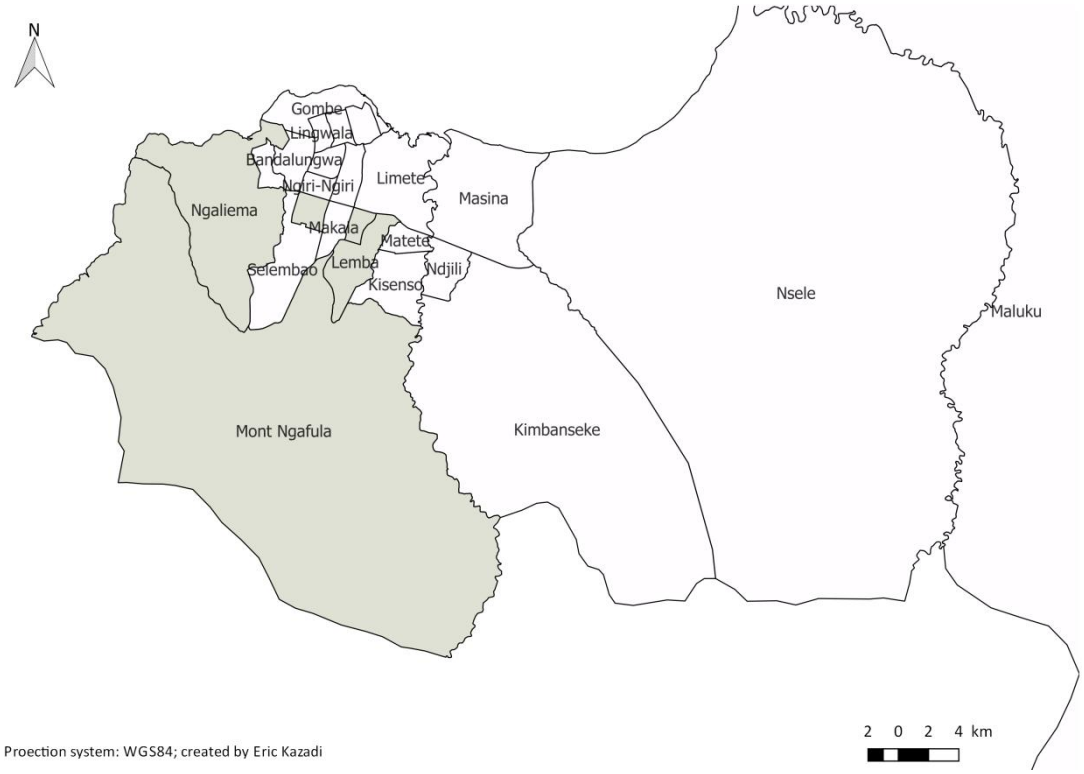

Figure 3.1. Map showing the *communes* of Kinshasa. Areas in grey indicate the *communes* where dogs were recruited for assessment of vaccine efficacy and duration of vaccine-induced immunity.

Table 3.1. *Communes* and *quartiers* of Kinshasa concerned by data and sample collection

N°	Study	Study area	Data or sample collection period
1	Dynamic of dog population	Four *quartiers* (Livulu, Righini, Ngomba-Kinkussa and Mongala) located in Lemba, Ngaliema and Bumbu *communes*	December 2017 to December 2018
2	Serological evaluation of efficacy of rabies vaccine	*Communes:* Lemba, Mont-Ngafula, Ngaliema, Bumbu and Ngaba	Day 0 and Day 30 (2018)
3	Serological estimation of duration of immunity provided by rabies vaccine	*Communes:* Lemba, Mont-Ngafula, Ngaliema, Bumbu and Ngaba	Day 0 and Day 8 (2018)

3.3.3. Data collection and analysis

a. Study of the dog population dynamics

A longitudinal demographic survey was conducted in the four selected *quartiers* from December 2017 to December 2018 (Table 3.1). Dogs were identified in dog-owning households that accepted to participate in the study. The following data were collected through three visits (December 2017, June 2018 and December 2018): (i) number of dogs owned, (ii) their sex and age (iii), reproductive history of females including litter size, (iv) fate of pups (i.e. kept, sold, given away or died), (v) number of newly arrived dogs, (vi) number of previously recorded dogs which were no longer present (died, sold, given away or stolen) at the next visit.

Collected data were used to estimate the following parameters: (i) the age of the third quartile (75%) of dogs, (ii) mean litter size and the pup mortality, (iii) reproduction rate (ratio between number females that had given birth and number of females recorded), (iv) birth rate (ratio between number of puppies born and number of recorded dogs), (v) growth rate, and (vi) the turnover rate of the population. The dog population growth rate was estimated as the natural log of the final population (P1) and the initial dog population (P0) or ln (P1/P0) (Caughly, 2004; Czupryna et al., 2016). The turnover rate was calculated as the ratio between new dogs and the final population (P1). New dogs included dogs that were born between the first and the third visit and those that were newly acquired.

b. Serological evaluation of the efficacy of rabies vaccine

The efficacy of rabies vaccine was tested in three age groups composed of 24 puppies (≤ 3 months of age), 37 juvenile dogs (3-12 months) and 22 adult dogs (>12 months). All dogs were reported as non-vaccinated by their owners and were vaccinated a single time using

Rabisin (Merial, 1IU/ml). Vaccines were purchased from a veterinary drugstore in Kinshasa. Venous blood was sampled the day of vaccination (D0) and one month later (D30). Serum was harvested after centrifugation and stored at -20°C. Anti-rabies antibody detection was performed by Sciensano National Reference Laboratory of Rabies in Belgium by use of Rapid Fluorescent Focus Inhibition Test (RFFIT), one of the WHO and OIE reference methods (Meslin et al., 1973; OIE, 2014).

Anti-rabies antibody titers were expressed in International Unit per milliliter (IU/ml) and 0.5 IU/ml of anti-rabies antibody was considered as the minimum protective titer according to the WHO recommendations (1992). The rabies vaccination was considered effective if the D30 titer was ≥0.5 UI/ml for dogs with D0 titer below the 0.5 IU/ml cut-off or if the D30 titer increased in dogs with starting titer ≥0.5 UI/ml. Anti-rabies antibodies was considered as undetectable if the titer was below 0.18 IU/ml.

Using the STATA software 11.0 (Stata Corp., college Station, Texas) a logistic regression model was used to explore if the percentage of protected dogs (titer ≥0.5 IU/ml) after vaccination differed between the three age categories (puppies, juveniles and adults).

c. Serological estimation of the duration of immunity in vaccinated dogs

The duration of immunity was estimated by determining serum antibody titer before (D0) and 5-8 days after (D8) administration of a booster vaccine in dogs whose last vaccination was performed since >1-2 years (n=31), >2-3 years (n=12) or >3-7.5 years (n=4). The RFFIT was used to quantify anti-rabies antibodies before and after the booster vaccination.

The proportion of dogs with protective titers (≥0.5 IU/ml) or without protective titers (<0.5 IU/ml) was calculated at D0 and D8. The anamnestic response was considered adequate in case of a titer rise at D8 of at least 0.5IU/ml. The duration of the immunity provided by rabies vaccine was estimated based on proportion of dogs with protective titer after the booster vaccination considering the time span since the last vaccination.

Using the STATA software 11.0 (Stata Corp., college Station, Texas) a logistic regression model was used to explore if the percentage of protected dogs (titer ≥0.5 IU/ml) following the booster vaccination was influenced by time span (years) between last and booster vaccination.

d. Vaccination compartmental model

The compartmental model aimed at estimating the rabies vaccination coverage in dog populations presenting low and high rabies transmission risk profiles. This model was adapted from the mathematical model published by Chidumayo (2018).

The present mathematical model was subdivided in three age compartments: (i) puppies (P: 0-3 months), (ii) juveniles (J: 3-12 months) and adults (A: >12 months). In addition, a vaccination compartment was created for juveniles only. Finally, 3 compartments of unprotected animals (J2, J3, A2) included unvaccinated animals and vaccination failure, and two rabies protection compartments (protected dogs) for juvenile (J1) and adult (A1) classes (Table 3.2). β1 and β2 were aging rates for transfer from compartments P to J and J to A respectively. p1 and p2 were respectively the vaccination proportion in puppies at 3 months and the proportion of juveniles dogs vaccinated each 6 months (3-12 months). Gamma (γ) was the vaccination success rate. Removal rates were denoted mu (μ) 1 to 3 for P, J and A compartments respectively (Fig. 3.2).

Table 3.2. Model core parameters

Model parameter	Symbol	Unit	Value	
			Area with low-roaming activity (<25%)	Area with high-roaming activity (≥75%)
Juvenile female proportion[a]	JFP	Dimensionless	0.49	0.44
Adult female proportion[b]	AFP	Dimensionless	0.40	0.38
Puppy removal rate (0-3 mo)	μ1	$Month^{-1}$	0.04	0.08
Juvenile removal rate (3-12 mo)	μ2	$Month^{-1}$	0.036	0.05
Adult removal rate (>12 mo)	μ3	$Month^{-1}$	0.016	0.026
Puppy maturation rate	β1	$Month^{-1}$	0.30	0.30
Juvenile maturation rate (3-12 mo)	β2	$Month^{-1}$	0.063	0.052
Juvenile reproduction rate (3-12 mo)	$\alpha 1$	$Month^{-1}$	0.009	0.018
Adult reproduction rate (>12 mo)	$\alpha 2$	$Month^{-1}$	0.019	0.033
Litter size	LS	Dimensionless	5	5
Proportion of vaccinated weaned puppies at 3 months of age	p1	$Month^{-1}$	0.8	0.8
Proportion of juvenile dogs (3-12 months) vaccinated each 6 months	p2	$Year^{-1}$	0.8	0.8
Estimated vaccination success[c]	γ	Dimensionless	0.9	0.9

Notes: mo months [a] Proportion in juvenile dogs [b] Proportion in adult dogs [c] Serological and challenge studies (Lakshmanan et al.,2006 ; Morters et al.,2015; Yangchen et al.,2019).

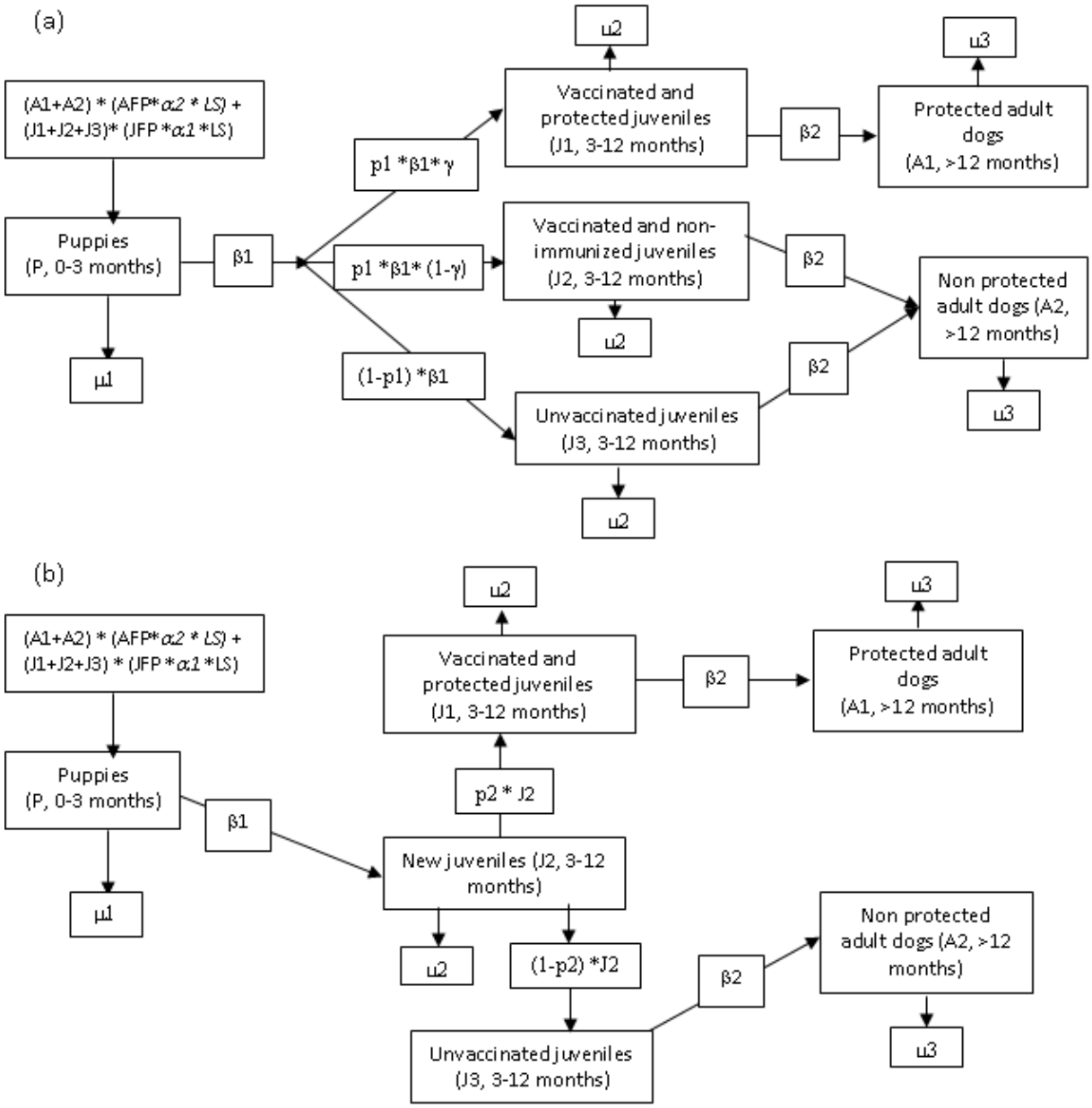

Figure 3.2. (a) Flow diagram of systematic vaccination of puppies at 3 months of age. The population was subdivided in three age compartments: (i) puppies (P: 0-3 months), (ii) juveniles (J: 3-12 months) and adults (A: >12 months). In addition, a vaccination compartment was created for juveniles only. Finally, 3 compartments of unprotected animals (J2, J3, A2) included unvaccinated animals and vaccination failure, and two rabies protection compartments (protected dogs) for juvenile (J1) and adult (A1) classes. β1 and β2 are aging rates for transfer from compartments P to J and J to A respectively. p1 is the vaccination rate in puppies at 3 months and p2 is the proportion of juvenile dogs vaccinated each 6 months. Gamma (γ) is the vaccination success rate. Removal rates are denoted mu (μ) 1 to 3 for P, J and A compartments respectively. (b) Flow diagram of annual vaccination of juvenile dogs (3-12 months). Juvenile dogs are transferred from unvaccinated subpopulation (J2) to vaccinated and immunized subpopulation (J1) at vaccination rate p2.

The following differential equations were used in order to test scenarios of:

a. Systematic vaccination of puppies at 3 months of age

$dP/dt = ((A1+A2) * AFP*\alpha 2 * LS) + ((J1+J2+J3) * JFP*\alpha 1 * LS) – (P * \beta 1) – (P * \mu 1)$ (1)

$dJ1/dt = ((p1*\gamma) * P * \beta 1) – (J1 * \beta 2) - (J1 * \mu 2)$ (2)

$dJ2/dt = ((1 - \gamma) * p1 * P * \beta 1) - (J2 * \beta 2) - (J2 * \mu 2)$ (3)

$dJ3/dt = ((1-p1) * P * \beta 1) – (J2 * \beta 2) - (J2 * \mu 2)$ (4)

$dA1/dt = (J1 * \beta 2) - (A1 * \mu 3)$ (5)

$dA2/dt = (J2 * \beta 2) + (J3 * \beta 2) - (A2 * \mu 3)$ (6)

b. Annual vaccination of juvenile dogs (3-12 months)

$dP/dt = ((A1+A2) * AFP*\alpha 2 * LS) + ((J1+J2+J3) * JFP*\alpha 1 * LS) – (P * \beta 1) – (P * \mu 1)$ (1)

dJ1= ifelse(times%%6<1, p2*J2, 0) - (J1* u2) – (J1 * β2)

dJ2= (P*β1) – ifelse (times%%6<1, p2*J2, 0)- (J2 * u2)

dJ3= ifelse(times%%6<1, (1-p2) *J3, 0) - (J3 *u2)- (J3 *β2)

dA1= (J1 * β2) - (A1 * u3)

dA2= (J3 *β2) - (A2 *u3)

Parameters are detailed in Tables 3.2.

Dog population values (number of puppies, juveniles and adults) were initialized by running the model 1000 times. Dog population stability was verified (plausible population growth, given birth and disappearance rates set). Population abundance values obtained after the convergence of the model were used to calculate the proportion of puppies, juveniles and adults. Initial values were calculated accordingly for the different dog categories (Table 3.3.)

Table 3.3. Age-related model parameters

Model parameters	Symbol	Area with low - roaming activity (<25%)		Area with high-roaming activity (≥75%)	
		Field observation	Model value	Field observation	Model value
Puppy proportion (0-3 months)	P	0.15	0.09	0.18	0.14
Juvenile proportion (3-12 months)	J1+ J2 +J3	0.15	0.21	0.28	0.35
Adult proportion (>12 months)	A1+A2	0.70	0.70	0.54	0.51

Abbreviations: P puppies, J1 vaccinated and non protected juveniles, J2 unvaccinated juvenile , J3 vaccinated and protected juveniles, A1 protected adults, A2 non protected adults

The following scenarios were tested: (i) Impact of complete vaccination stop in high and low risk populations presenting an initial vaccination coverage of 60% and 80%; (ii) impact of systematic puppy vaccination in high and low turnover rate populations with an initial vaccination coverage of 0%, 24% (coverage recorded in high risk populations in Kinshasa; Kazadi et al 2020) or 60%, and (iii) impact of biannual pulse vaccination of juvenile dogs (3-12 months) in low and high turnover rate populations with an initial coverage of 0%, 24% (coverage recorded in high risk populations in Kinshasa; Kazadi et al 2020) or 60%.

3.4. Results

3.4.1. Dog population dynamics

The characteristics of the dog population are displayed according to low or high roaming activity and subsequent rabies transmission risk in Table 3.4. The dog turnover rate in areas with high-roaming dog activity was twice (36%) as high as in areas with low-roaming dogs' activity (17%). Similar differences were found for reproduction rates in adult (39% versus 23%) and juvenile (22% versus 11%) females. The annual disappearance rate was 9% in area with low-roaming activity and 40% in area with high dog roaming activity.

The dog population was young: 75% (third quartile) of identified dogs in areas with low and high turnover rate were less than 3 years old. The median age of dogs did not differ between areas ($p=0.24$) and was respectively 14 and 24 months in high and low-roaming activity areas. Adult dogs aged 5 years represented less than 10% of identified dogs (Fig. 3.3). However, the age structure differed between areas: puppies (0-3 months) and juvenile dogs (3-12 months) made up 46% of dog population in high risk and high turnover rate settings, whereas both fractions made up of 30% of dog population in low risk and low turnover rate (Fig 3.4).

Table 3.4. Characteristics of the investigated dog population in Kinshasa between 2017 and 2018

Demographic parameters	Letter code	Formula	Area with low- roaming activity(<25%)	Area with high-roaming activity (≥75%)
Quartiers	-	-	Righini and Ngomba-Kinkussa	Livulu and Mongala
Number of visited dog-owning households				
2017	a		112	190
20118	b		112	190
Number of recorded dogs				
2017	c		153	280
2018	d		178	270
Number of disappearing dogs between 2017 and 2018	e		16	109
Number of newly enrolled dogs	f		31	99
Growth rate	g	ln(d/c)[a]	0.15	-0.03
Turnover rate	**h**	**f/d**	**0.17**	**0.36**
Number of adult females (>1 year)	i		42	51
Number of adult females that had given birth between 2017 and 2018	j		10	20
Adult reproduction rate	k	j/i	0.23	0.39
Mean litter size of adult female	l		5	5
Number of juvenile female (<1 year)	m		18	27
Number of juvenile females that had given birth between 2017 and 2018	n		2	6
Juvenile reproduction rate	o	n/m	0.11	0.22
Mean litter size of juvenile females	p		4	4
Number of puppies born	q	l*j+p*n	58	124
Birth rate	r	q/d	0.32	0.45
Disappearance rate	s	e/d	0.09	0.40

[a] Calculation of growth rate according to Caughly (2004) and Czupryna et al. (2016)

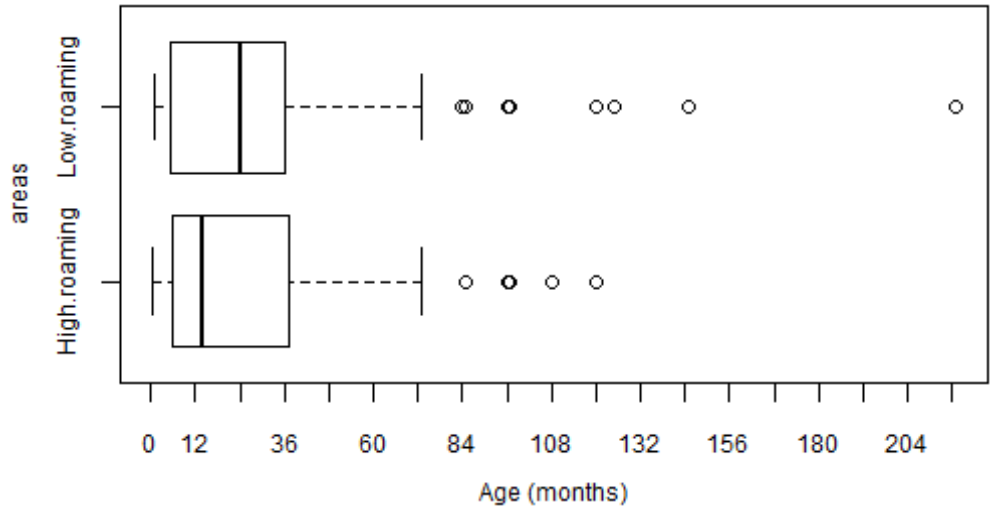

Figure 3.3. Age distribution of identified dogs in areas with low (n=178) and high (n=280) proportion of free-roaming dogs

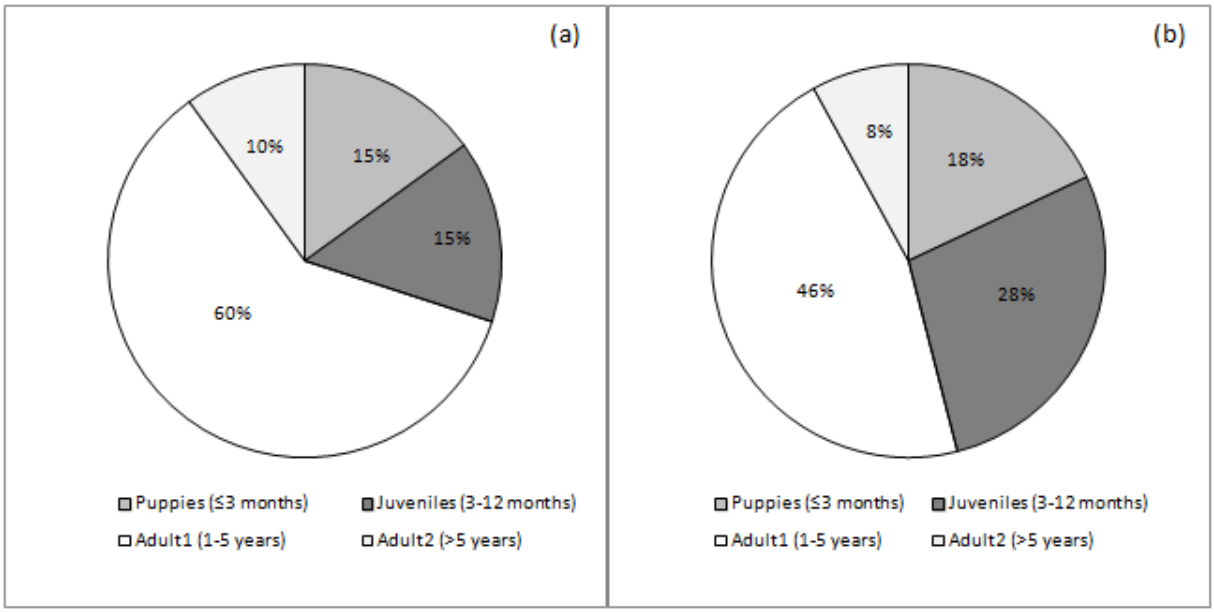

Figure 3.4. Age structure of dog population respectively in (a) low risk and low turnover rate (n=178) and (b) high risk and high turnover rate (n=280)

3.4.2. Serological evaluation of the efficacy of rabies vaccine in puppies, juvenile and adult dogs

At D0, the proportion of recruited dogs with protective anti-rabies antibody titers was 47% (39/83). This surprisingly high proportion varied from 41 to 55% and did not differ between age groups (p=0.89) (Fig, 3.5).

At D30, the proportion of protected dogs (titer ≥0.5 IU/ml) equaled 98% (81/83) and ranged from 94% to 100% without differing between age groups (p=0.24) (Fig. 3.5). Vaccination failure occurred in one puppy and one juvenile dog.

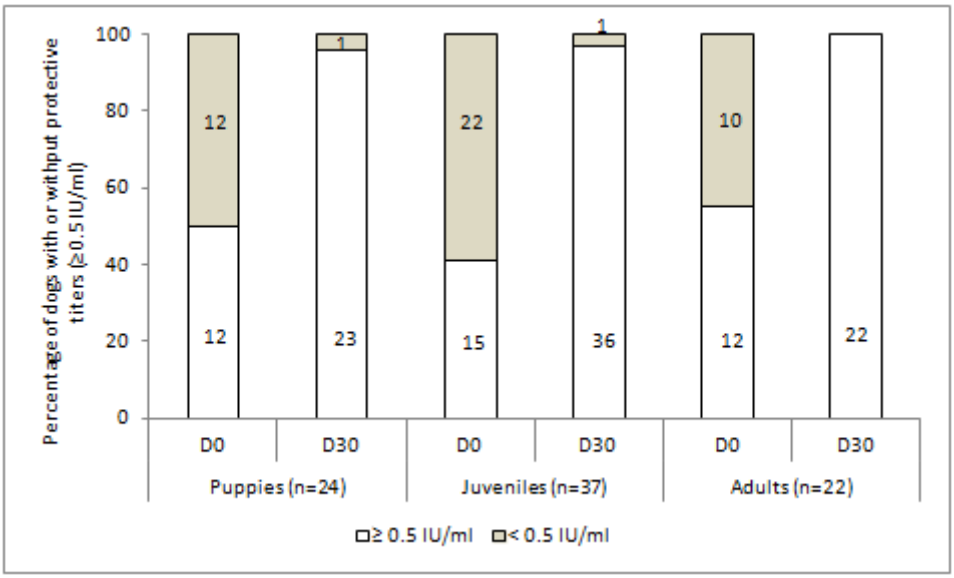

Figure 3.5. Proportion of puppies, juvenile and adult dogs presenting protective anti-rabies antibodies titers (≥0.5 IU/ml) before (D0) and after (D30) rabies vaccination. No group-related differences between vaccine responses were found (p=0.24).

3.4.3. Serological estimation of the duration of immunity in vaccinated dogs

Before booster vaccination at D0, the proportion of dogs with protective titers equaled 74% (35/47) and did not differ in function of time span since last vaccination (p=0.051; Fig. 3.5).

At D8, the proportion of dogs with protective titers equaled 96% (45/47) without differing between dog groups (p=0.5; Fig. 3.5). Two dogs vaccinated 1-2 years before the booster administration did not display a titer increase compatible with an anamnestic response.

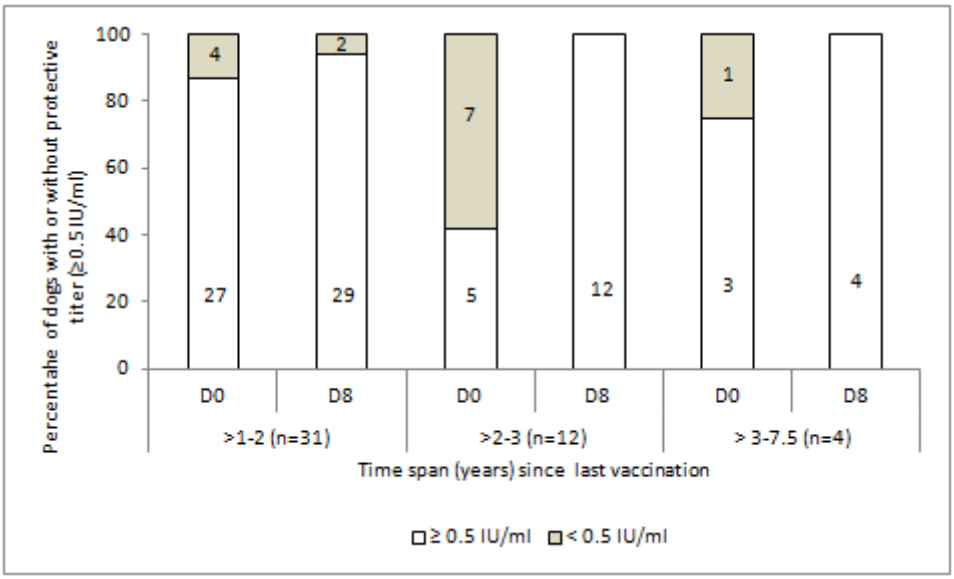

Figure 3.6. Proportion of dogs presenting protective antibody titers (≥0.5 IU/ml) before (D0) and after (D8) rabies booster vaccination in function of time span since last vaccination (>1-2 years; >2-3 years; >3-7.5 years). No anamnestic response differences were found between groups (p=0.5).

3.4.4. Vaccination compartmental model

In case of vaccination stop (scenario1), the proportion of immunized dogs drops in function of canine turnover rate. By considering an initial proportion of immune dogs of 80%, the critical threshold of 40% would be reached after 27 months in low risk and low turnover (17%) populations whereas a high risk and high turnover (36%) population would reach the threshold after 18 months. By considering an initial protection of 60%, the 40% threshold would be respectively reached after 15 and 10 months (Fig. 3.7).

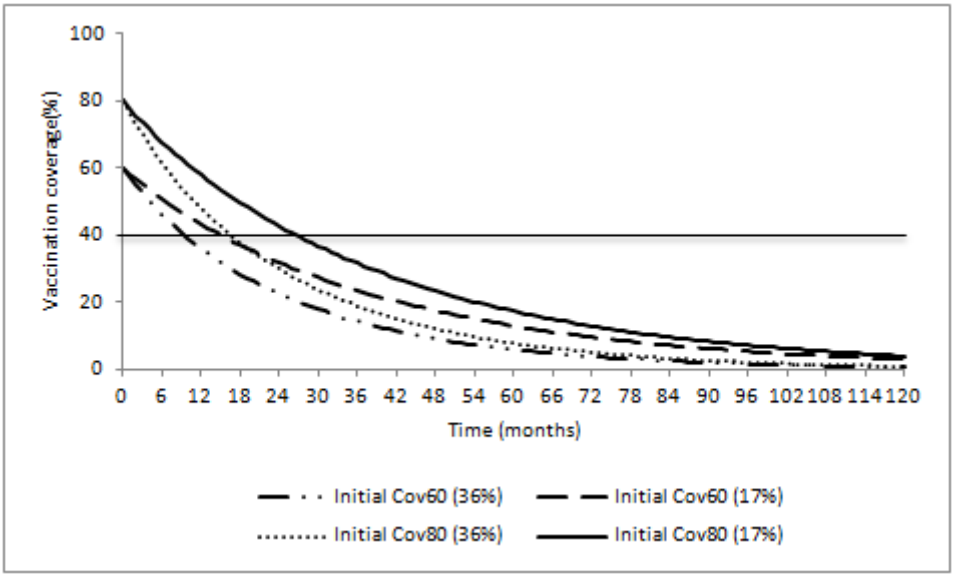

Figure 3.7. Effect of dog turnover rate on rabies vaccination coverage in absence of yearly vaccination campaigns. The model predicts the time (in months) needed for waning of rabies herd immunity below the critical threshold of 40% considering an initial herd immunity of 80% or 60% and a dog turnover rate of 17 or 36%.

When vaccinating systematically 80% of puppies at 3 months of age, the initial coverage of 60% remained above the threshold control in both low and high dog turnover populations. By considering an initial coverage of 24% (observed rate in Kinshasa), the critical (40%) and control (60%) thresholds would be reached respectively within 13 and 73 months in high turnover population and within 19 and 80 months in low turnover population. By considering an initial coverage of 0%, both thresholds would be reached respectively after 25 and 86 months in high turnover population whereas 37 and 98 months would be required in low turnover population (Fig. 3.8).

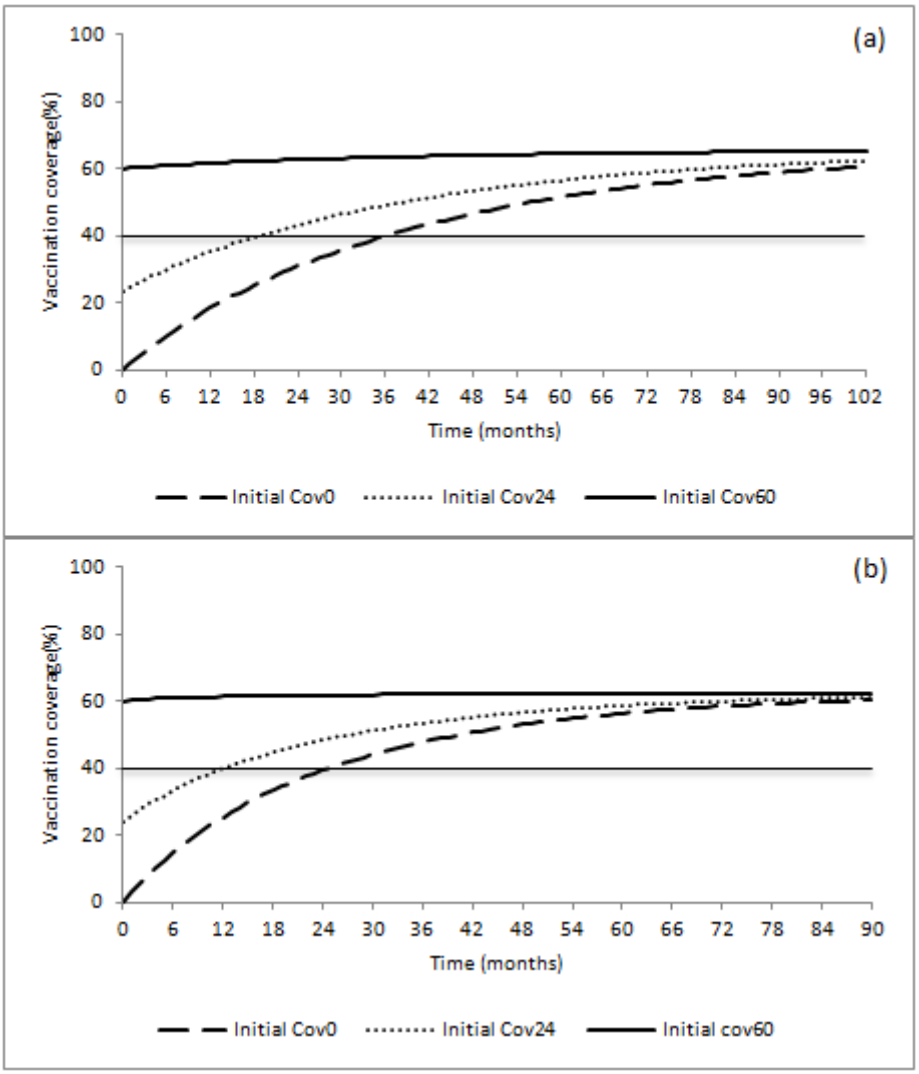

Figure 3.8. Impact of systematic vaccination of puppies (at 3 months of age) in (a) low and (b) high dog turnover populations with initial vaccination coverage of 0%, 24% (coverage recorded in high risk populations in Kinshasa; Kazadi et al., 2020) or 60%. The vaccination success rate (γ) was 90.

When 80% of juvenile dogs (3-12 months) are vaccinated each 6 months, the initial coverage of 60% remains above the critical threshold in both low and high dog turnover rate populations. By considering an initial coverage of 24% (observed rate in Kinshasa), the coverage increase gradually and reaches the critical threshold (40%) respectively within 31 and 37 months in low and high turnover populations. By considering an initial coverage of 0%, the coverage reaches the critical threshold after 49 months in both low and high turnover populations (Fig. 3.9).

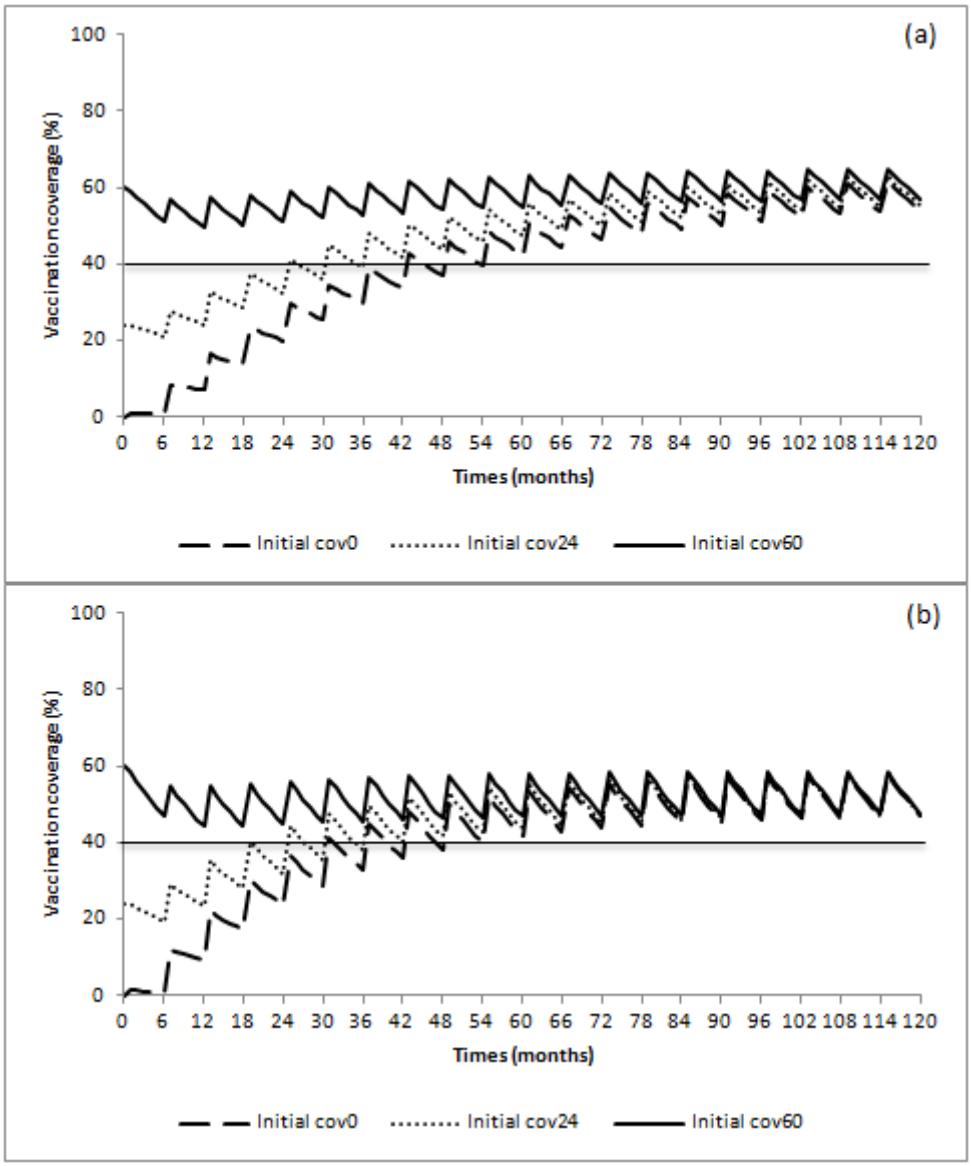

Figure 3.9. Impact of biannual pulse vaccination of juvenile dogs (3-12 months) in (a) low (17%) and (b) high turnover rate population (36%) with initial vaccination coverage of 0%, 24% ((coverage recorded in high risk populations in Kinshasa; Kazadi et al., 2020) and 60%.

3.5. Discussion

The present study aimed at proposing a risk-based vaccination scheme by considering (i) the dynamics of canine populations with different risk profiles for rabies transmission and (ii) the efficacy and duration of a serologically-detectable immunity in response to vaccination and revaccination tested under field conditions.

The turnover rate was used as the main indicator of dog population dynamics. Indeed, it represents the fraction of new (susceptible) dogs which influence the vaccination coverage

kinetics. However, as new dog-owning households in surveyed areas were not included in the study, the turnover rate could be slightly underestimated.

Our study evidenced the link between the dog-keeping practices and the turnover rate. It was high (36%) in area with high roaming activity (>75% of dogs are free-roaming) and low (17%) in low roaming activity (<25% of dogs are free-roaming). Similar differences were found for birth rates (45% versus 32%) and disappearance rates (40% versus 9%). Indeed, in high roaming population, dogs were free to mate, whilst most of them were not castrated or spayed. In addition, juvenile and adult dogs were in danger of being stolen or sold to the dog meat consumers. The mortality of puppies before weaning was also high. Similarly, high turnover rates of 37% and 50% were reported in settings with high roaming dog activity, respectively in Tunisia and Kenya (Seghaier et al., 1999; Kwoba et al., 2019). In contrast, the low turnover rate (17%) estimated in low roaming activity settings was close to the average turnover rate of 20% reported in Europe and North-America where good dog-keeping practices are observed (Jibat et al., 2015).

The primo-vaccination success rate and duration of a serologically-detectable immunity in response to revaccination were two jointly addressed topics for answering the following research question: "is the single vaccination sufficient to provide a lifetime protection against rabies?" Indeed, beside the turnover rate, the vaccine characteristics such as its ability to provide a lifetime protection was a key parameter of our vaccination model as discussed below. Our results showed that regardless of the age groups and prevaccinal titers, the vaccine was effective in 98% (81/83) of vaccinated dogs. Surprisingly, 47% (39/83) of dogs possessed prevaccinal protective titers (≥0.5 IU/ml). Similarly, in Tunisia and Brazil, were respectively 32% and 42% of dogs with unknown historical of vaccination had protective titers (Seghaier et al.,1999; Fernades et al., 2017).

In our study, the origin of prevaccinal antibodies should be clarified in order to avoid biased calculation of the vaccination success rate, mainly in puppies (≤3 months). It is highly likely that owners did not report the true vaccination status of their dogs given that the vaccine was gratuitous, whilst it cost up to 20 USD (Kazadi et al., 2017). This may explain the origin of prevaccinal protective titers mainly in juvenile and adult dogs. But, in puppies of less than 3 months of age, anti-rabies antibodies may originate either from their mother or from previous vaccination. Antibodies may also originate from rabies infection. This assumption seems unlikely, given that recruited puppies appeared to be in good health during

the paired blood collection period (day 0 and 30). It is likely that puppies (2-3 months of age) with prevaccinal protective titers were previously vaccinated against rabies. There are three givens in this argument: (i) the age of puppies (2-3 months), while it is established that maternally-derived antibodies decline significantly from weaning (10 weeks of age) (Day et al., 2016), (ii) the high serologically vaccination success rate of 100% (12/12), while it is assumed that high level of maternal antibodies hamper antibody production and (iii) the strong post-vaccination response (titers: 3.4 to 10 IU/ml), although high prevaccinal titers that ranged from 0.5 to 4.7 IU/ml (Table S3.1). Other serological studies reported the proportion of 23% to 26% respectively in Bhutan and Tunisia, but without providing the antibody origin (Seghaier et al., 1999; Yangchen et al., 2019). In Sir Lanka, none of clearly identified puppies (1.5-3 months) from vaccinated dams had prevaccinal protective titers (Pimburage et al., 2017). In brief, our vaccination was considered as booster vaccination for 50% (12/24) of puppies with prevaccinal protective titers, whereas it is considered as primary-vaccination for other 50% (12/24) of puppies without prevaccinal protective titers. Thus, the assessment of vaccine efficacy in terms of serological response to primo-vaccination showed that the vaccination success rate was 92% (11/12). One puppy failed to seroconvert for unknown reason. The success rate ranging from 78% to 100% was reported in puppies by other serological studies (Cliquet et al., 2003; Kennedy et al., 2007; Morters et al., 2015; Wallace et al., 2017; Tasioudi et al., 2018).

It is important to note that the serological vaccination success rate is strongly associated to the time span between vaccination and blood test, the composition of vaccine used (antigen content, adjuvant used, mono or polyvalent) and it storage conditions (Zanoni et al., 2010; Kennedy et al., 2007). It was reported that the primo-vaccination success rate is usually above 92% when blood was collected within 1 to 2 months after vaccination and drops up to 78% when antibodies are quantified 6 months after vaccination (Cliquet et al., 2003; Minke et al.,2009; Van Gucht et Le Roux, 2010; Pimburage et al.,2017). In addition, when rabies vaccine is administered in conjunction with core vaccines (polyvalent vaccines), the success rate ranges from 78% to 91%. But when used as monovalent vaccine, its serological success rate is above 93% (Cliquet et al., 2003; Van Gucht et Le Roux, 2010). Furthermore, inactivated and adjuvanted (aluminum salts) rabies vaccines such as Rabisin (1IU/ml, Merial) provided a high success rate (≥96%) including in puppies (Minke et al.,2009, Morters et al.,2015, Nokireti et al.,2017, Yangchen et al.,2019). However, these vaccines are not thermostable, especially on freezing storage conditions under which the

adjuvant (aluminum salt) precipitates and thereby loses efficacy (Kartoglu and Milstien, 2014; Kumru et al., 2014). In contrast, a field study demonstrated that inactivated and adjuvanted vaccine (Novibac, Intervet) provided a similar vaccination success rate when it was stored at room temperature (up to 30^{o}C) during several months, as when it was stored as recommend by manufacture (2 to 8^{o} C) (Lankester et al., 2016). Thus, in order to reduce potential biases associated with storage conditions, the vaccine used in the present study (Rabisin, Merial) was purchased in drugstore (D) into which the vaccine was relatively-well stored according to manufacture advices (2 to 8^{o} C) (Fig. S3.1). Taken together, above evidences strongly suggest that using a good quality vaccine may provide a serological success rate of at least 90% in puppies of below 3 months of age.

Furthermore, there is a conflict between regulation of some countries and scientific evidences about the duration of immunity provided by rabies vaccine in dogs. Indeed, in some countries such DRC, annual revaccination of dogs against rabies is still mandatory (Arrêté N^{o}SC/151/BGV/MIN/AGRI & DR/SMI/2016) assuming that the duration of immunity last about 1 year, whereas in other countries where the law was modified or updated, 3 years is the minimum duration of immunity provided by the same vaccine such as Rabisin (Day et al., 2016). Our results suggest that the duration of immunity provided by inactivated vaccine (Rabisin) last up to 3 years given that 96% (45/47) vaccinated since >1 to 7.5 years, display a rapid and strong serological response within 5 to 8 days upon revaccination. Only small number (2) of dogs did not display a rapid serological response within 5-8 days (Table S3.2). There are two possibilities: either these dogs were not previously vaccinated (Brown et al., 2016) or they were vaccinated in spite of slow serological response. Our results are in line with other several serological and challenge studies which reported that the duration of immunity provided by rabies vaccine could last between 3-7 years in dog and up to 14 years in humans (Lakshmanan et al.,2006 ; Schultz, 2006 ; Roth and Spickler, 2010; Malerczyk et al., 2007; Moore et al.,2015; Day et al.,2016).

Our study highlighted that rabies vaccine is effective in puppies of below 3 months of age and that it provides a long-term protection (≥ 3 years). However, these results may not directly lead to conclude that a single vaccination provides a lifetime protection, hence the reference to other studies. Précausta et al. (1985) evidenced that the protection against a pathogenic strain of rabies virus was excellent (97%) 3 years after a single vaccination. Lakshmanan et al. (2006), demonstrated that 88% of vaccinated once at 3 months of age with an inactivated vaccine survived more than 3 years later against a virulent challenge In captive

African wild dogs, anti-rabies antibodies were still detectable over 3 years after the single vaccination (Connolly et al.,2015). Aubert (1992) claimed that the survival chance against a pathogenic strain of rabies virus was the same for dogs with detectable or no detectable neutralizing antibodies at moment of challenge, provided that dogs without detectable neutralizing antibodies had successfully seroconverted after vaccination. Therefore, reassured by above evidences and the short life expectancy of observed dog populations (≤3 years for 75%) as in most of African countries (Jibat et al.,2015), we suggest that a single dose of a good quality of rabies vaccine provides a life protection. This key evidence is the core parameter of vaccination strategy based on the systematic vaccination of puppies at weaning age (≥2 months) as discussed below.

Despite demonstrated positive impact of regular annual mass vaccination strategy for rabies control and elimination (Kitala et al., 2002; Hampson et al. 2007, Hampson et al.,2009, Global Alliance for Rabies Control, 2015), its implementation in rabies endemic countries is challenged by several factors such the lack of adequate resources. Yet, it appears that the risk of rabies transmission varies within settings or towns (Kazadi et al., 200), thereby making high risk zones as high-priority settings for action against rabies. This is the first level of improving the efficiency of vaccination program.

By modeling the vaccination scheme, two coverage thresholds: 40% considered as the critical threshold under which large rabies outbreak may occur in dog population (Coleman & Dye, 1996; Hampson et al.2009) and ≥60% referred to a threshold for effective rabies control (Cleveland et al., 2003; WHO, 2013). In particular, the critical threshold was used as a reference point for determining the frequency of mass vaccination campaign in low and high risk zones. Thus, by considering the turnover rate, when the initial coverage is 80%, the mass vaccination campaign may be implemented no more each 1.5 years in high risk and turnover rate (36%) population, whereas its can be safely implemented each two years (biennial) in low risk and low turnover rate (17%). With a free of charge mass vaccination campaign and community support, the vaccination coverage of 80% is reachable as reported in Zambia, Chad, Tanzania and Indonesia (Debalogh et al., 1993; Kayali et al.,2003; Kaare et al.,2009; Léchenne et al., 2016; Arief et al.,2017). Furthermore, our study confirming clearly the influence of turnover rate on kinetic of coverage and therefore the frequency of mass vaccination. Thus, ignoring the influence of turnover rate may lead to non-effective vaccination program. This was the case in Tunisia where without taking account of turnover rate (37%), the country decided to shift from annual to biennial mass vaccination scheme

from 1982 till 1992. However, six year later (1988), large rabies outbreaks occurred and the country decided to abandon biennial scheme in favour of annual mass vaccination campaigns (Seghaier et al., 1999). Notwithstanding this example, the biennial scheme may be implemented in low risk and low turnover rate population (≤17%) and may provide monetary savings ranged from 32 to 42% (Bilinski et al. (2016). Although the biennial scheme seems theoretically efficient, it cannot be safely implemented in most of urban and rural settings in endemic countries due to high turnover rate as the consequence of poor dog-keeping practices and poverty (Seghaier et al., 1999; Kwoba et al., 2019; Kazadi et al., 2020).

In these poor and high risk zones, we propose the systematic vaccination of puppies at weaning (2-3 months of age), which is viewed as an alternative to the annual mass vaccination campaigns. Indeed, between two annual vaccination campaigns, the proportion of susceptible dogs mainly composed of puppies and juvenile dogs which were puppies at mass vaccination moments, may reach 50% (Fig 3.4) of dog population, thereby justifying the rapid decrease of coverage in high risk and high turnover rate (36%). Yet, vaccine is effective in puppies and provides a lifetime protection taking account the serological success rate of ≥90%. These results constitute the first strong epidemiological reason to opt for systematic vaccination of puppies at 2-3 months of age. The model showed that the systematic vaccination of puppies (2-3 months of age) prevents the decrease of initial coverage. To the contrary, it contributes to gradually growth of coverage. Ideally, this scheme must be implemented after a mass vaccination campaign. In the extreme case (no financial resources for mass vaccination implementation), the systematic vaccination of 80% of puppies will contribute to prevent large rabies outbreaks within 13 and 25 months by increasing initial coverage from 24% and 0% to 40% (critical threshold) in high risk and high turnover rate (36%) population.

The second epidemiological reason is the high accessibility of puppies for parenteral vaccination. Indeed, the accessibility is one of the success key of vaccination strategies, given that it is affected by several factors such as the dog–human relationships, the dog owner's perception about vaccination and the distance between the vaccination posts and households (Kaare et al., 2009; WHO, 2013; Jibat et al., 2015; Gibson et al., 2016). Studies reported that 15 to 40% of adult dogs are usually not bringing to vaccination posts either because they were aggressive or because they had run away (Lembo et al., 2010; Kayali et al., 2003; Minyoo et al., 2015; Muthiania et al., 2015). In contrast, young dogs (<12 months) and specially puppies

(≤3 months) usually remain near their homestead and seem therefore easy to catch and to handle to vaccination posts (Minyoo et al., 2015, Muthiania et al., 2015, Arief et al.2017). However, some dog-owners continue to perceive that puppies are too young to be vaccinated and could refuse to bring them to vaccination posts (Minyoo et al., 2015, Muthiania et al., 2015, Kazadi et al., 2017; Kazadi et al., 2020). This great issue for the success of monthly systematic vaccination of puppies could be addressed by an appropriate advertising or awareness strategy, by the significant reduction of vaccination cost and mainly by boosting community engagement.

The third and last second reason is economic, based on assumption of economic benefits associated to inclusion of puppies in vaccination program (Kaare et al., 2009). Indeed, the average mass dog vaccination cost per dog is US$ 4.03 (Min: US$ 1.56- Max: US$ 11.33). It was estimated that more than 60% of the mass vaccination campaign budget is spent on covering the vaccinator costs (perdiem, transportation…) given the time (1-3 months) dedicated each year by vaccinators for vaccination campaign (Kayali et al., 2006; WHO, 2013; Wallace et al, 2017,). We believe that given the small number of puppies aged of 3 months to be vaccinated each month , the rabies vaccination shall be easily integrated in routine activities of public animal health workers (veterinarians and para-veterinarians) which will dedicated just few days (1 or 2 days) for vaccination of available weaned puppies at low geographic levels. This vaccination strategy may theoretically provide monetary savings and seems to be more sustainable. However, due to poor veterinary infrastructures across the country, the storage of rabies vaccine could be a challenge (Niang and Denormandie, 2008; Diop et al., 2012; Ministère de la Pêche et de l'Elevage, 2017). This issue can be addressed through the intersectoral collaboration (One Health approach) between the Public health and Veterinary sectors given that most of health centers are equipped with refrigerators and solar panels or fuels for good storage of human vaccines (PATH,2016). Conversely, the intersectoral collaboration it will be needed also for better assessment of the impact of this vaccination strategy on the rabies incidence.

Finally, the mandatory systematic vaccination of puppies at 3 months of age will be a great opportunity for gradual identification of dogs at the veterinary services as required by law since 1918 in DRC (Royal Decree of 22 January 1918).

Furthermore, the biennial pulse vaccination of juveniles (3-12 months) could be an acceptable alternative if the systematic vaccination is facing sociocultural barriers that may

impact the accessibility of puppies to parenteral vaccination or logistical challenges such the lack of cold chain for ensuring a good storage of vaccine. In addition, the biennial vaccination strategy may enhance the chance of catching up dogs that were not previously reached, contrary to the strategy of unique vaccination of dogs at weaning. However, only field cost-effectiveness studies may help to determine the most cost-effective vaccination strategy for resource-poor settings.

3.5. Conclusion

The study evidenced the link between the dog-keeping practices and the turnover rate, which influences the coverage kinetics. Hence, the needs to put in place a setting-specific vaccination scheme. The turnover rate was high in high risk and high roaming dog population, thereby leading to rapid decrease of vaccination coverage. In contrast, in the same high turnover rate settings, when dogs are systematically vaccinated at 3 months of age), herd immunity is preserved even in high turnover areas, thereby confirming the epidemiological needs for inclusion of puppies in vaccination program. Theoretically, the systematic vaccination of puppies at weaning seems to be efficient and more appropriate rabies vaccination strategy for rabies control at source in resource-poor and endemic countries, despite high turnover rates.

Chapter 4. Overview of the intersectoral collaboration in the management of rabies in Democratic Republic of the Congo

Understanding local epidemiological factors is a prerequisite to design the most appropriate and effective rabies control strategy. Local risk factors and risk zones were identified in our fist study (Chapter 2). Accordingly, the established risk zones were used in the development of a risk-based vaccination scheme that shall be supported by local rabies network for its success. The objective of this preliminary survey was to describe the existing rabies surveillance and control networks in DRC and to assess the level of interaction between the concerned professional sectors. The study revealed that institutions of the wildlife sector were not involved in rabies management, contrarily to medical and veterinary institutions which make up the rabies surveillance and control networks in DRC. Despite the fact that medical and veterinary institutions are implanted across the country, the study showed that the collaboration between them was inadequate at operational and strategic levels. The structural weaknesses and the lack of political will have emerged as the main causes of the lack or the inadequate collaboration between sectors.

Preliminary survey

4.1. Abstract

Rabies is endemic in Democratic Republic of the Congo (DRC). As it is a zoonosis, its control requires a strong and effective intersectoral collaborative approach, which may contribute to a more efficient use of available resources. The objective of this preliminary survey was to describe the existent rabies network in DRC and to assess the level of interaction between sectors using the stakeholder analysis method. A total of 17 institutions including governmental (public) and non-governmental (professional bodies and international agencies) institutions that were assumed to be involved in rabies management in DRC were considered. Governmental institutions were national and subnational institutions of medical, veterinary and wildlife sectors. Fifteen key informants from 15 institutions were available to participate in the study and were interviewed. All key informants had a good understanding of One Health. They stated that intersectoral collaboration between medical, veterinary and wildlife sectors is the right approach for rabies management in DRC. The study highlighted the fact that institutions of wildlife sectors are not involved in rabies network in DRC, mainly because of the lack of surveillance system of wildlife diseases. The rabies network is made up mainly of institutions of medical and veterinary sectors. However, the collaboration between medical and veterinary sectors is weak, despite the existence of human and animal disease surveillance systems and the countrywide implantation of medical and veterinary institutions. Resources and data are not shared between both sectors either because they are not available or due to the lack of legal collaborative framework. The structural weaknesses and the lack of political will have emerged as the main barriers to strong collaboration among stakeholders that are involved in rabies management in DRC. However, a further study with a wider range of stakeholders and respondents should be conducted in order to reach the sampling saturation and to refine the present findings through the triangulation method.

4.2. Introduction

Rabies is endemic in Democratic Republic of the Congo (DRC) and in all bordering countries namely Angola, Burundi, Central African Republic, Republic of Congo, Rwanda, South Sudan, Tanzania, Uganda, and Zambia (Repetto, 1932; Courtois et al., 1964;, Makumbu, 1977; Georges, 1982; Bula and Mafwala, 1988; Swanepoel et al., 1993; Ali, 2002; Muyila et al.,2014; Twabela et al., 2016; Pieracci et al., 2017; Muleya et al., 2019). In addition, it is a zoonosis, that infects all mammal species including humans (Rosset, 1985). Thus, its control requires an intersectoral collaboration (Léchenne, 20015).

In light of the above, we propose this preliminary survey that aimed at describing the existent rabies surveillance and control networks in DRC and assessing the interaction level between sectors or institutions using the stakeholder analysis method.

4.3. One Health and stakeholder analysis

One Health is a strategic framework for reducing risks of infectious diseases at the animal-human-ecosystem interface by promoting the cooperation, communication and coordination among sectors or areas of expertise (Mackenzie and Jeggo, 2011). It is also promoting the use of available resources by integrating the disease control strategies (FAO, 2014). Yet, rabies is a zoonotic disease requiring the collaboration between actors or sectors for an efficient control (Léchenne, 2015). These actors can be also called stakeholders, a term that, in a One Health framework, may cover the ultimate beneficiaries (human, animals and environment) as well as the governmental and non-governmental organizations (government ministries, research institute, professional bodies, civil society, local and international agencies) that work to protect them against diseases (Mazet et al.,2014). More widely speaking, stakeholders may be considered as anyone who impacts a project or is impacted by this project.

Furthemore, the stakeholder analysis consists of methods allowing to (i) identify stakeholders, (ii) categorize stakeholders, and (iii) investigate relationships between stakeholders (Reed et al., 2009). It was the main method used in the present preliminary survey.

4.4. Preliminary survey

This preliminary survey was carried out from April to May 2017, in Kinshasa, the capital of DRC that hosts subnational (provincial), national and international institutions. Indeed, Kinshasa is simultaneously a Province and the capital of DRC. The stakeholder analysis method was used but limiting the stakeholder concept to formal organizations that were assumed to be involved in the rabies management in DRC. These could be subnational (provincial) and national governmental institutions and local or international non-governmental institutions. The first listed stakeholders were governmental institutions that belong to the medical, veterinary and wildlife sector. Then, non-governmental stakeholders were listed through the snowball sampling method, which is a convenience sampling method for finding research subjects in a context were no exhaustive list of potential interviewees with their relevant characteristics are available. Starting from first key-informants, each interviewee will propose to the research team other potential stakeholders according to their understanding of the research question and their knowledge of the relevant actors. Sampling continues until data saturation (Naderifar et al., 2017). In the present case, this survey being preliminary, no saturation was sought and for each stakeholder listed, one key informant was identified and contacted by phone to make an appointment for the interview.

The role of stakeholders in relation with rabies and their perceptions about One Health were collected through semi-structure interviews, guided by a check-list (S4.1). The semi-structured interview is a qualitative method that needs a trust-based relationship between the investigator and the interviewed (Imbert, 2010). The interview tacked the actor's perception about the OH approach, the motivation, the predisposition to work in collaboration with other sectors and the possible challenges. The check-list included the following items: (i) the institution role in relation to rabies management, (ii) the rabies control actions taken, (iii) understanding of OH approach, (iv) involvement in OH approach and difficulties or challenges encountered, (v) OH initiatives taken in relation with rabies, (vi) proposed strategies for making the OH approach a reality in the rabies management, and (vii) expectations from implementation of OH approach. Key informants responses were summarized in a table.

The status and level of collaboration between stakeholders in relation with rabies management, was assessed (Table 4.1), based on a grid covering on key elements of effective intersectoral collaboration proposed by the Food and Agriculture Organization (FAO), World

Health Organization for Animal Health (OIE) and World Health Organization (WHO) (FAO/OIE/WHO, 2012). This tool included nine key elements that were divided in supporting and operational elements. These include : (i) availability of the intersectoral rabies program, (ii) availability of the legal collaborative framework (iii) data sharing, (iv) human resource sharing, (v) material sharing, (vi) financial resource sharing, (vii) organization of multisectoral workshop, (viii) joint planning of rabies control activities, and (ix) joint implementation of rabies control activities. The first two elements were supporting elements and considered as key elements. They were binary variables (yes/no). The last seven key elements were operational criteria. They were also binary variables (yes/no). In case of existing collaboration, the frequency of collaboration was specified. This frequency was graded as (i) regular, (ii) irregular or (iii) rare. Based on these criteria, four possible levels of interaction were established between the three public major stakeholders: (i) strong interaction (ii) moderate interaction (iii) weak interaction and (iv) no interaction. Finally, a relational diagram was developed based on the level of interaction between stakeholders. This assessment of level of collaboration was particularly focused on governmental stakeholders.

In particular, with the purpose of assessing the data-sharing between sectors, reported and notified rabies data were compared. "Reporting is the act of a clinician /veterinarian /laboratory informing the local health/veterinary agency of a suspected or confirmed case of a disease. Notification is the process of the local health/veterinary agency informing the national health/veterinary agency and to the relevant international organizations such as World Health Organization (WHO) and World Health Organization for Animal Health (OIE)" (Canine Rabies Blueprint, 2017).

Table 4.1. The ranking table of interaction level between stakeholders which are supposed to be involved in the rabies management in DRC

N^0	Key elements	Interaction level ranking			
		Strong	**Moderate**	**Weak**	**Non-existent**
1	Availability of the intersectoral rabies programme	Yes	Yes	No	No
2.	Availability of the legal collaborative framework	Yes	Yes	No	No
3.	Data sharing	Yes/regular	Yes/irregular	Yes/rare	No
4.	Human resource sharing	Yes/regular	Yes/irregular	No	No
5.	Material sharing	Yes/regular	Yes/irregular	No	No
6.	Financial resource sharing	Yes/regular	Yes/irregular	No	No
7.	Organization of multisectoral workshop	Yes/regular	Yes/irregular	Yes/rare	No
8.	Joint planning of rabies control activities	Yes/regular	Yes/irregular	Yes/rare	No
9.	Joint implementation of rabies control activities	Yes/regular	Yes/irregular	Yes/rare	No

4.5. Main observations

Seventeen governmental and non-governmental organizations that were assumed to be involved in rabies management in DRC were listed. Two were not available for interviews (country office of the WHO and the municipality of Kinshasa).

In relation with rabies, stakeholders of the veterinary sector (5) perceived their role as that of surveillance and control of rabies in domestic animals on the one hand, and that of prevention of human rabies (treatment of bite victims) on the other hand. Stakeholders of medical sector (3) described their role as one of surveillance and prevention of rabies in humans. Stakeholders of wildlife sector (2) reported having no role in the control of rabies. Furthermore, both animal health professional bodies stated that their role was limited to encourage their members (veterinarians and paraveterinarians) to participate in rabies control activities. Interviewed international agencies (FAO, OIE, CDC) claimed to provide technical support to governmental institutions which are involved in rabies control (Table 4.2).

All key informants from listed subnational and national governmental institutions (10) had a good understanding of the One Health approach. They claimed that OH approach was the only way for the control of zoonotic diseases such as rabies. However, in practice did not work collaboratively for rabies control, except some rare activities. They argued that the lack of legal collaborative framework, the lack of logistic and financial resources and the lack of surveillance system (as stated particularly by the wildlife institutions) were the main barriers that impede the intersectoral collaboration. Furthermore, the leadership conflict between

medical and veterinary sectors was particularly mentioned by veterinary sector as another barrier against the intersectoral collaboration. They think that political will is the only way to make the OH approach a reality. The expectations were the creation of a national program for the fight against zoonotic disease, the capacity building of medical, veterinary and wildlife workers and surveillance systems and the standardization of rabies data collection tools (Table 4.3).

Table 4.2. Role of institutions supposed to be involved in the rabies management in DRC

N^0	Institution	Role in relation with rabies
Veterinary sector		
1	Direction de Production et Santé Animale **(DPSA)**	Planning and implementation of rabies activities in animals across the country
2	Service Quarantaine Animale et Végétale **(SQAV)**	Checking rabies vaccination certificate of pets at borders
3	Laboratoire Vétérinaire **(LVC)**	Diagnosis of animal rabies cases
4	Bureau Provincial de Santé Animale **(BPSA)**	Planning and implementating of rabies activities at rovincial) level
5	Office de vaccination et de contrôle rabique **(OVCR)**	Treatment of human bite victims
		Tracing of suspected animal rabies cases
		Supervision of mass vaccination campaigns
Medical sector		
6	Direction de Lutte des Maladies **(DLM)**	Rabies surveillance (national level) in humans
		Treatment of human bite victims
7	Institut National des Recherches Biomédicales **(INRB)**	Diagnosis of human and animal rabies cases
8	Bureau de surveillance **(B4)**	Rabies surveillance (local/provincial) level
		Treatment of human bite victims
Environnement/Wildlife health sector		
9	Institut Congolais pour la Conservation de la Nature **(ICCN)**	Management of wildlife animals in protected areas
10	Direction de Conservation de la Nature **(DCN)**	Management of wildlife animals in unprotected areas
Professional bodies		
11	Association des Médecins Vétérinaires du Congo (**AMVC**)	Encourage veterinarians to join in fighting against rabies in the country
12	Association des techniciens vétérinaires du Congo (**ATVCO**)	Encourage paraveterinarians (veterinary nurses) to join in fighting against rabies in the country
International agencies		
13	Food and Agriculture Organization **(FAO)**	Technical support of public institutions
14	World Organization for Animal health **(OIE)**	Collecting, analyzing and disseminating national rabies data
15	Centers for Disease Control and Preventions **(CDC)**	Financial and technical support of the field epidemiological and training program (FELTP)/ One Health program

Table 4.3. Key informant perceptions of OH approach

N0	Institutions	Understanding of OH approach	Involvement in OH approach and challenges encountered	OH initiatives taken in relation with rabies	Proposed strategies for making the OH approach a reality	Expectations from OH approach
1	DPSA	This approach ensures that each actor of network plays its role in collaboration with other actors	My institution is a member of the national One Health committee. But, with respect to rabies, no action has been taken due to lack of financial resources.	No OH initiative in relation with rabies has been taken.	Funding of OH committee and providing a legal framework for regulation of collaboration among actors.	Training of stakeholders for awareness for their specific role.
2	LVC	Integral application of prevention, detection and response against zoonotic diseases by involving medical, veterinary and wildlife sectors	Yes	Rare meetings held at DLM and planning rabies mass campaigns.	Creation of a legal collaborative framework for all actors involved in rabies management that establishes duties and responsibilities of actors.	Extension of legal collaborative framework for rabies control to fight against other emerging and re-emerging zoonosis.
3	SQAV	The fight against rabies must include experts from medical, veterinary and wildlife sectors, dog owners and policy makers	My institution is theoretically involved in OH. But, in practice, each institution works in vacuum, whereas normally results and actions should be shared among actors	Rare OH initiatives such as the rabies mass vaccination of dogs, multidisciplinary workshop.	Collaboration between institutions requires first of all a political will. Then, the creation of a multidisciplinary institution like the national program to combat rabies.	Creation of a multidisciplinary institution like the national program to combat rabies
4	BPSA	Close collaboration between medical and veterinary structures.	Yes, but with major difficulties such as: leadership conflict between medical and veterinary actors, lack of financial resources and structural collaborative framework.	Jointly planning with medical sector of mass vaccination campaign in Kinshasa.	Creation of a multidisciplinary structure called national program for fight against zoonosis. This program must be lead by the veterinary sector given that animals are the main source.	Creation of a multidisciplinary structure called national program for fight against zoonosis.

Abbreviations: Direction de Production et Santé Animale (DPSA), Laboratoire Vétérinaire Central (LVC), Service de Quarantaine Animale et Végétale (SQAV), Bureau de Production et Santé Animale (BPSA).

N0	Institutions	Understanding of OH approach	Involvement in OH approach and challenges encountered	OH initiatives taken in relation with rabies	Proposed strategies for making the OH approach a reality	Expectations from OH approach
5	OVCR	A new spirit that put together experts from several fields for control of zoonosis.	Yes, but with major financial and logistic difficulties	OVCR was created in response to intersectoral to meet a need for integrated rabies control.	Creation of national program for rabies control that must ensure an open and sincere intersectoral collaboration and provide personal capacity-building.	Collaboration between medical and veterinary sector for rabies control.
6	DLM	Multidisciplinary, intersectoral and collaborative approach that addresses risks at human, animals and wildlife interfaces.	There is no formal OH team. Our institution works informally with veterinarians if zoonotic epidemics occur such as during the avian influenza outbreak in 2003.	Jointly planning with veterinary sector of mass vaccination campaign of pets.	Creation of a legal collaborative framework between medical, veterinary and wildlife sectors.	Creation of a legal collaborative framework between medical, veterinary and wildlife sectors. Harmonization of rabies data collection tools
7	INRB	Human health is closely linked to health of pets such as rabies.	No. Communication and funding gaps	My laboratory includes veterinary and medical workers..	Support the surveillance and control of rabies in animal population.	Support the intersectoral collaboration to ensure that human and animals are healthy.
8	B4	Close collaboration between medical and veterinary structures for control of zoonotic diseases.	No.	Jointly creation of rabies task force in 2012 which is not functional.	Institutionalization of rabies control at the highest level of the state.	Promotion of collaboration between medical and veterinary sectors.
9	DCN	Prevention, detection and response to rabies require involvement of all actors.	Yes	We are in an embryonic stage in relation with disease surveillance and control activities.	Implementation of data flow system	Wildlife sector is less involved in health activities. OH approach is an opportunity for capacity building of actors of wildlife sector.
10	ICCN	Rabies affects human, pets and wildlife, hence the interest of having human, animal and wildlife rabies surveillance systems.	Yes	No	Intensification of the contacts between stakeholders in order to determine how to improve the management of rabies.	Revitalization of the national One Health committee.

Abbreviations: Direction de Production et Santé Animale (DPSA), Laboratoire Vétérinaire Central (LVC), Service de Quarantaine Animale et Végétale (SQAV), Bureau de Production et Santé Animale (BPSA), Office of vaccination and rabies control (OVCR), Direction de Lutte contre la Maladie (DLM), Institut National de Recherche Biomédicale (INRB), Bureau de surveillance (B4), Institut Congolais de Conservation de la Nature (ICCN), Direction de Conservation de la Nature (DCN)

According to criterion for assessing the level of collaboration across sectors (Table 4.1), only the 7th and 8th criteria were rarely met by medical and veterinary institutions. The following jointly activities were: (i) the establishment of One Health rabies task force in Kinshasa which is non-operational, (ii) the organization of multidisciplinary workshop on rabies that has been initiated by the Food and Agriculture Organization (FAO, 2012), (iii) the planning of rabies mass vaccination campaign of pets, and (iv) the holding of a formal weekly multisectoral surveillance meeting at local and central levels by medical sector (Table 4.3).

The relational diagram (Fig 4.1) showed that the wildlife health sector was completely disconnected from both medical and veterinary sectors regarding rabies management. In contrast, the collaboration between medical and veterinary sectors was weak or inadequate.

No data-sharing was noted between institutions of wildlife health sectors. Rabies data were regularly shared inside institutions of medical and veterinary sectors according to data flow (Fig. 4.1), except, the "Service de Quarantaine Animale et Végétale (SQAV)". This institution which belongs to the veterinary sector is particularly involved in rabies control at border crossing through the control of rabies status of pet animals. The comparison data on human bite victims and human clinical rabies cases reported from 2013 to 2016 by the "Bureau Provincial de Santé Animale (BPSA)" (veterinary sector) and the "Bureau de surveillance (B4)" of medical sector revealed the discrepancy between data reported by both provincial institutions (Fig. 4.2). The figure S4.1 shows the flow data in medical and veterinary surveillance systems.

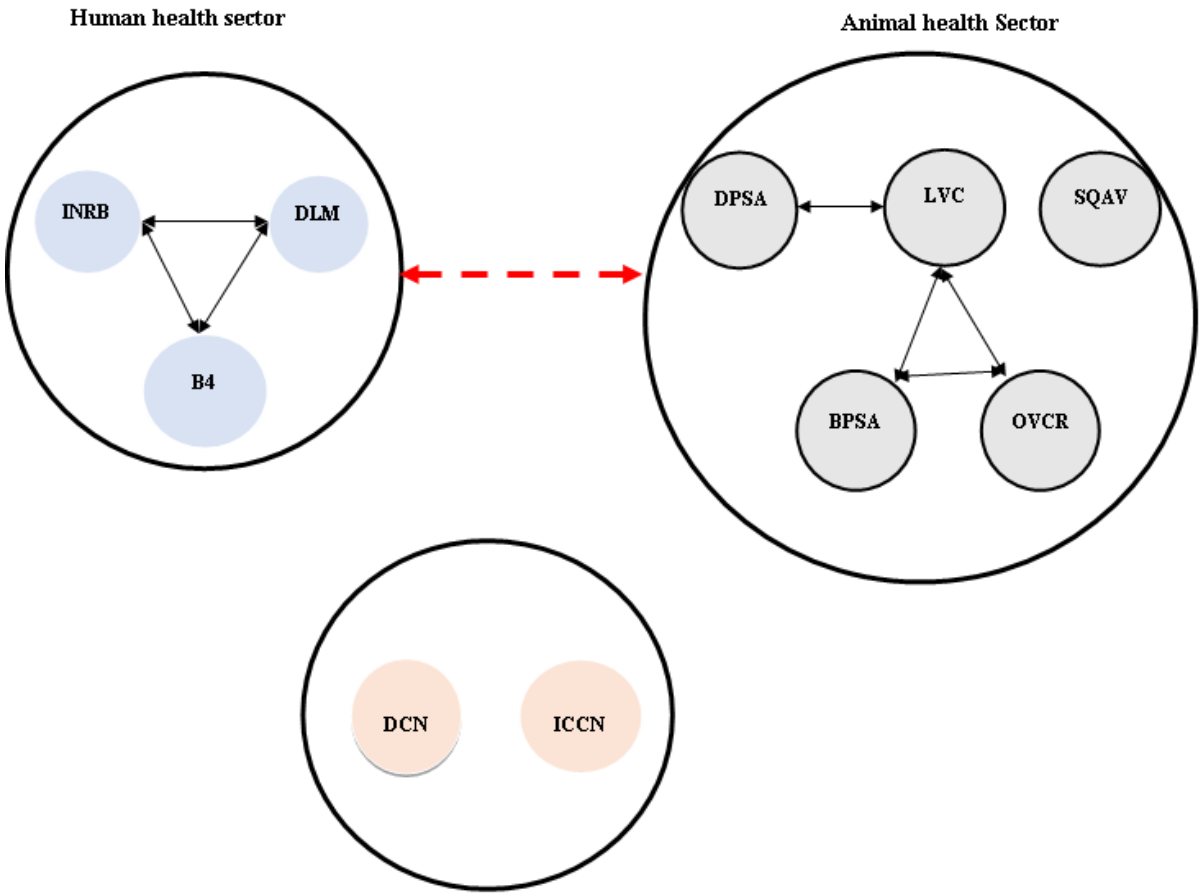

Figure 4.1. The interaction between the stakeholders of governmental (public) sector of DRC. An arrow was associated to each level of interaction: (i) continue arrow (strong interaction), (ii) dotted arrow (moderate interaction) and (iii) broken arrow (weak collaboration). Inside the sector, a continue double headed arrow indicates the rabies data-sharing.

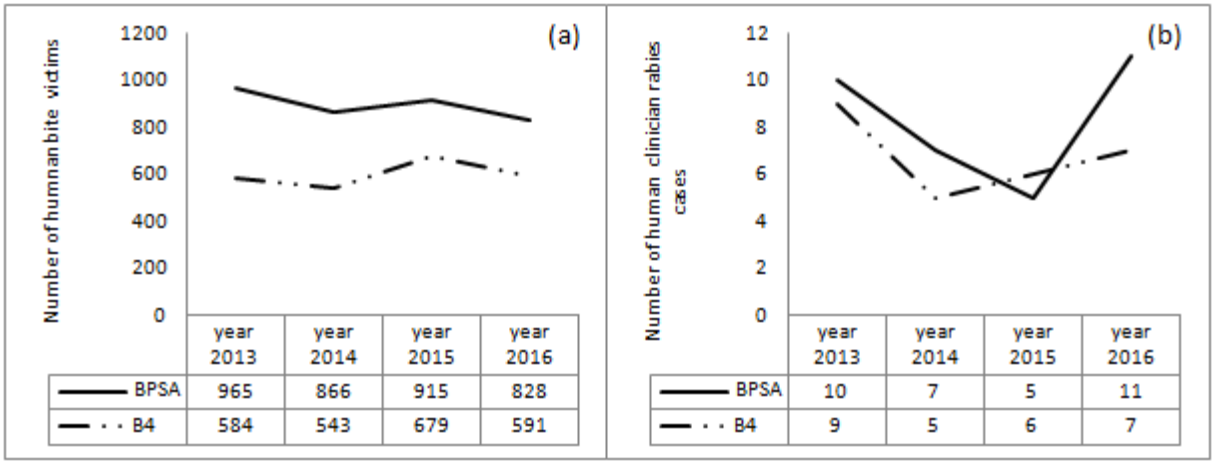

Figure 4.2. Reported (a) human bite victims and (b) human rabies cases from 2013 to 2016 by two provincial institutions namely BPSA (veterinary sector) and B4 (medical sector)

4.6. Discussion and perspectives

This preliminary survey revealed that the One Health concept was not a new concept for governmental stakeholders. However, its application in relation with rabies management was still a theory given the lack or low level of collaboration between these governmental stakeholders (Fig. 4.1). Several factors would contribute to limit the translation of the One Health concept from theory to practice. They can be grouped in two categories: structural and political constraints. They are discussed below.

According to expert opinions, the data-sharing between sectors is the crucial factor for success or failure of a OH initiative (Cleaveland et al., 2014, Global Alliance for Rabies Control, 2015; Rüegg et al., 2018). This preliminary survey showed a lack of sharing and harmonization of rabies data between the medical and veterinary sectors (Fig 4.2), notwithstanding the fact that the medical sector holds weekly multisectoral surveillance meetings at local and national levels, which can be considered as a framework for sharing rabies data. The lack of legal collaborative framework was reported by stakeholders as the main reason. We think that beside the lack of legal collaborative framework, the structure and functioning of medical and veterinary surveillance systems (Fig. S4.1) could contribute to lack of data-sharing. Both factors could be considered as structural factors.

Indeed, in DRC, the veterinary sector must report both animal and human rabies data according to reported role of this sector in rabies control. All biting or suspected rabid animals (pet animals) should be placed in quarantine for 21 days in public pounds. Unfortunately, they are not usually isolated either because the information is not relayed to local veterinary service on time or due to lack of appropriate infrastructures (pounds) for the animal isolation. As an alternative, a weekly close home-based follow up of biting pet animals may be applied by official veterinarians. These animals are euthanized (poisoned) once clinical signs of rabies occur. But, at the grass root level, there are no adequate logistical resources for collection, packaging and shipping of samples to veterinary laboratories. In addition, the country has only 3 veterinary laboratories located in 3 Provinces (Kinshasa, Haut-Katanga, Nord-Kivu), while DRC is a huge country made up of 26 Provinces and with poor infrastructures. We must note that rabies sample analysis including the trepanation for collection brain sample costs between 50-100 USD. Taken together, above reasons explained why few animal laboratory rabies data are available. For example, from 1980 to 2017, the Central Veterinary Laboratory of Kinshasa received only 249 suspect animal rabies samples, resulting in annual average of 7

samples. The public veterinary service had submitted only 30 samples during the same period, representing a low participation level of 12%, resulting in an annual average of 1 (Fig. S4.2). The average annual number of suspect animal samples submitted for laboratory rabies testing was extremely low compared to those annually analyzed in other developing and rabies endemic countries such as Kenya (128), Ethiopia (350) and Bolivia (392) (Widdowson et al.,2002; Deressa et al., 2010; Bitek et al., 2019). The poor performance of veterinary services was reported in a majority of Sub-Saharan African countries due to lack of financial resources since colonial era and structural adjustments periods (Sidibe, 2003; Niang et al., 2008; Diop et al.,2012; Bardosh et al.,2017; Ministère de Pêche et Elevage, 2017). Therefore, animal rabies data to share with other sectors are usually not available.

On the other hand as mentioned above, the veterinary sector is generating human rabies data (Fig. S4.1a). Indeed, most of public veterinary clinics located in provincial capitals (Kinshasa, Kananga, Mbuji-Mayi,etc) had created formal (e.g. OVCR in Kinshasa) or informal rabies centers where human victims receive post-exposure prophylaxis (PEP).This PEP is administered by nurses or physicians, thereby making these formal or informal centers as illustration of multisectoral framework. However, rabies data are annually reported from bottom to the top of veterinary surveillance system jointly with other administrative reports of Ministry of Agriculture, Fisheries and Livestock, whereas in medical sector, data are weekly reported from bottom to the top of surveillance system (Fig. S4.1.b). This discrepancy in frequency of reporting data could be a major reason of lack of data-sharing, despite the existence of weekly intersectoral surveillance meeting organized by the medical sector.

Furthermore, rabies data from medical sector are also under-reported due to inadequate surveillance system and lack of rabies centers across the country (Muyila et al., 2014). Therefore, health facilities are the primary data collection sites of rabies data. Yet, 30% of health facilities were not integrated into medical surveillance system. The case definition list of diseases with epidemic potential under surveillance was not available in 40% of health facilities. If it was available, the case definition of rabies was not yet included systematically given that rabies was just recently (2012) listed among disease under surveillance (Ministère de la Santé Publique, 2011 Kazadi, 2014; Ministère de la Santé Publique, 2017). The wildlife sector was not yet involved in rabies management because of lack of functional surveillance system. The priority of institutions of wildlife sector is the protection of wild animals against poaching (Ministère de l'Environnement et Développement Durable, 2017; Institut Congolais pour la Conservation de la Nature, 2017).

The second constraint seems to be the lack of political will as reported by stakeholders. Indeed, DRC is a country with a strictly centralized administration. National ministries are the decision-making centers and they play a critical role in the formulation and implementation of policies for disease control. Thus the failure to take sufficiently into account the true rabies burden by high-level policy makers may contribute to a lack of political will in relation with rabies control. Accordingly, rabies is still officially ranked among Neglected Tropical Disease in DRC (Uniting to Combat NTDs, 2016). Indeed, it is well known that diseases are often prioritized according to those that are most visible or perceived as a public health threat. Failure to command attention leads to a lack of political commitment and resources required the implementing an effective elimination strategy. The lack of rabies data seems to be the fundamental challenge of rabies prioritization in most of Sub-Saharan African (SSA) countries (Fahrion et al., 2016; Canine Rabies Blueprint 2017; WHO, 2017; Scott, 2017; Taylor, 2017).

As a direct consequences of this lack of political will in relation with rabies control, the disease is neglected (Uniting to Combat NTDs, 2016) and keeps killing mainly in poor settings where both veterinary rabies vaccine cost (20 USD) and post-exposure prophylaxis cost (350USD) are not affordable for majority of people who are mostly living under the poverty threshold in DRC (Moummi et al., 2010; Muyila et al., 2014; Bodjick,2016; Kazadi et al., 2017 ; Mpoyo et al.,2018; Kazadi et al., 2020). Yet, the close communication including data-sharing between the human and animal health sectors could significantly reduce the overuse of PEP (Lembo et al., 2010; Léchene et al., 2015). The country benefits from medical and veterinary structures which are well established in several parts. In addition, since 2005, the community-based surveillance of human diseases was implemented across the country (Mawazo, 2013).

Yet, the political will may result in creation of a National Program for the Prevention and Fight against Zoonotic Diseases (NPFZD) by the Prime Minister decree as in some African countries such as Uganda, Nigeria and Cameroon where success stories were reported (Nzietchueng et al., 2013; Okello et al., 2014). It may also result in the establishment of a legal and mandatory collaborative framework at local and national levels. This program or legal framework should clearly define the role of each actor, the process and framework of sharing of data, human, material and financial resources and establish the chain of command (Bogel et al.,1992; Léchenne et al.,2018;). Hence, this would also appear as needed in the context of leadership conflicts as were mentioned here by interviewed actors. Additionally,

the program or legal framework provides opportunity for donor investments in rabies elimination (Global Alliance for Rabies Control), as is currently the case of several diseases such malaria, HIV/AIDS, tuberculosis, etc.

In conclusion, this preliminary survey highlighted that the rabies surveillance and control network in DRC is made up mainly of institutions of medical and veterinary sectors. Institutions of wildlife sectors are not included in rabies network. However, the collaboration between medical and veterinary sectors was weak, notwithstanding the existence of surveillance system of human and animal diseases. The structural weaknesses and the lack of political will have emerged as major barriers to strong collaboration among governmental stakeholders. However, a further study with a wider range of stakeholders and respondents should be made in order to reach the sampling saturation and to refine the present findings through the triangulation method. The future study must include (i) high political decision-makers (ministries) as stakeholders in order to investigate reasons for the lack of political in relation with rabies control and (ii) research institute (universities), civil society, local and international non-governmental organizations for purpose of specifying the role of each one in rabies control in DRC. In addition, a field study should be conducted at area-scale in order to (i) assess the acceptability of the integrated approach for rabies prevention and control by the medical, veterinary and wildlife workers and the community, and (ii) assess the cost-effectiveness of OH in relation with rabies (e.g. use of post-exposure prophylaxis).

Chapter 5. General discussion

This chapter discusses the key findings obtained from chapters 2, 3 and 4, which theoretically lead to improve the efficiency of rabies control program in poor resource settings. The strengths and weaknesses of each key finding are addressed.

5.1. The epidemiological value of using the dog ecology survey as a tool for the field establishment of rabies risk map

The study highlighted that poor dog-keeping practices and low vaccination coverage were the main factors of rabies transmission in dog population. These factors may lead to the stratification of risk zones. They were estimated using the dog ecology survey method. Thus, it is important to establish the epidemiological value of this method as tool for the field establishment of risk map.

Dog ecology survey is thought to be useful estimate accurate data such as the human to dog ratio (HDR), dog density, age and sex structure of dog populations, dog keeping-practices (dog roaming activity) and vaccination coverage (FAO, 2014). Different methodologies can be used to estimate these parameters: (i) mark-recapture, (ii) street-count method or direct observation of dogs on public properties, and (iii) household survey (WHO, 1987; Kitala et al., 2001; Kayali et al., 2003; WSPA, 2007; Kazadi et al., 2020).

Household survey is more appropriate in areas where dogs are not registered by the national services (WHO, 1987) and where few numbers of free-roaming dogs are feral as reported in Zimbabwe, Tanzania, Chad and DRC (Butler and Binghame, 2000; Cleaveland, 2014; Kazadi et al., 2020). Despite the fact that it generates accurate and useful data, it appears to be costly (Townsend et al., 2013). In DRC, the household survey can be transformed into relatively cheap method by involving existing volunteer community health promoters or "relais communautaires". Investigators should visit dog-owning households between 8 am and 6 pm (lowest roaming time) in order to maximize the chance to see find dogs and collect accurate data on dog characteristics (age, breed…) mainly in *quartiers* with high proportion of free-roaming dogs (WHO, 1987). Dog-owning households have to be selected randomly to achieve high representative level of the dog population in an area.

If these conditions are met, the household survey may help to estimate accurately the dog density, roaming activity and vaccination coverage of dog population, which are considered as risk factors of dog rabies transmission. Despite the controversy about the role of dog density role as risk, it was established that rabies is persisting when dog density was ≥5

dog/km^2 in endemic regions of Africa (Foggin, 1988; Brooks, 1990, Cleaveland and Dye; 1995; Kitala et al. 2002). The weight of dog density as risk factor of rabies transmission should be contextualized in combination with the data on dog-keeping practices (abundance of free-roaming dogs) given that both factors influence the contact rate (Kazadi et al.,2020). In contrast, there is evidence that the free-roaming associated with low vaccination coverage are risk factors for rabies transmission (Kazadi et al., 2020). Indeed, the absence of a physical barrier as illustrated in Fig. S2.1 evidenced that owned dogs are either partially or permanently free-roaming, that consequently increase their risk of coming into contact with rabid dogs. Once exposed (bitten by a rabid dog), the risk of developing rabies infection is very high for a dog especially if it is not vaccinated against rabies. However, the risk of rabies transmission in dog population depends on herd immunity; hence the existence of two coverage thresholds: 40% considered as the critical threshold under which large rabies outbreak may occur in dog population (Coleman & Dye, 1996; Hampson et al.2009) and ≥60% referred to a threshold for effective rabies control (Cleveland et al., 2003; WHO, 2013). These coverage thresholds have been established either empirically from observations on the relationship between vaccination coverage and rabies incidence (Korns, 1948) or theoretically based on estimation of basic reproduction number of rabies (R_0) (Coleman & Dye, 1996). They can be adapted according to local field data if the influence of the dog density and rate contact on the basic reproduction number of rabies is clearly established (Coleman & Dye, 1996; Bengo et al., 2002). In practice, the vaccination status of owned dogs may be assessed by considering owner's report (history of vaccination and time of last vaccination) or the vaccination certificate (if available). In the absence of vaccination certificate, mis-classification of vaccination status of dog may occur. On the one hand, people might fear to declare they owned dogs that were not vaccinated given that rabies vaccination is mandatory since 1938 in DRC (Royal Decree of 01 April 1938). On the other hand, dog owners may falsely report that their dogs are not vaccinated in order to benefit from gratuitous vaccination. Our serological results showed that only 9% (12/132) of reported vaccinated dogs for several years had no detectable anti-rabies antibody (<0.18 IU/ml), further supporting the reliability of dog owner reports.

Based on these results, we strongly suggest the use of household survey for data collection to estimation of dog density, roaming activity and vaccination coverage. We also suggest the use of the developed tool for local risk assessment and risk zone stratification using collected data related to the three risk factors and their corresponding thresholds (Table

2.1). In addition, if data about dog bite or rabies incidence are available, they can be used for improving the stratification of risk zones. For instance, high risk zones are usually characterized by high abundance of free-roaming dogs and low vaccination coverage as the consequence of poverty (Kazadi et al., 2020).

Given the link established between the risk zones and their socio-economic levels, beside the household survey, it may be appropriate in the future to use geographic tools such as earth observation (EO), geographic information systems (GIS) and field observations of areas (different from household survey) for urbanization classification at area-scale and stratification of risk zones. For instance, the earth observation tool can help to map slum areas or the presence of solid waste which increase the exposure of all individuals in communities to vector-borne and zoonotic diseases (Thomson et al., 2019).

5.2. Age at first vaccination and systematic puppy vaccination strategy

Assuming that a single vaccination at 3 months protects the animal for at least 3, the outcomes of vaccination compartmental models showed that the implementation of systematic vaccination of puppies at weaning seems to be a more efficient and appropriate rabies vaccination strategy for rabies control in resource-poor and endemic countries, despite high turnover rates. Three months of age is just an average. Puppies can be successfully vaccinated before they reach the age of 3 months as found in the present study, despite the persistence of controversies about the efficacy of rabies vaccine in puppies below 3 months of age.

Indeed, rabies vaccine manufactures advice not to vaccinate puppies below 3 months of age assuming that they have an immature immune system or that their immune response to rabies vaccine may be inhibited by maternal antibodies (Barrat et al., 2001, Morters et al., 2015). In addition, puppies less than 3 months old are less mobile and present less chance to be bitten by a rabid dog (Minyoo et al., 2015, Muthiania et al., 2015, Arief et al.2017). As a consequence, puppies below 3 months of age are excluded from rabies vaccination program. In contrast, the World Health Organization recommends the inclusion of this category of dogs in vaccination program, especially in rabies endemic countries (WHO, 2013).

This contraction is indeed nourished by conflicting evidence that can be found in published studies. Précausta et al. (1985) showed in a serological study that in presence of maternal antibodies, the production of anti-rabies antibodies was inhibited in puppies vaccinated at one month of age with an inactivated rabies vaccine. In addition, Aghamo et al.

(1990), found that the serological response of puppies (4-12 weeks) to vaccination with live vaccine was different whether puppies were from rabies vaccinated-or unvaccinated bitches. The antibody production was strong and earlier (from 4 weeks of age) in puppies from unvaccinated bitches, whereas it was delayed and strong only at 10 weeks in puppies from vaccinated bitches, although the fact that maternal antibodies decrease substantially from 6 weeks of age. In contrast, other serological studies showed the opposite and suggest that the inactivated rabies vaccine was effective in puppies aged from 10 days to 3 months in the presence or absence of maternally derived antibodies (Barat et al., 2001; Morters et al., 2015; Yangchen et al., 2019). Our own results (Fig 3.5) demonstrated that the rabies vaccine was effective in 96% (23/24) of puppies, although the presence of antirabies antibodies which could either originate from their mother or from previous vaccination that was not reported to inestigators. In addition, the only puppy which failed to seroconvert had no detectable anti-rabies antibody titer (≤0.18 IU/ml) before vaccination, contrasting the assumption of interfering maternal antibodies. Finally, a challenge study of puppies aged of 2 weeks (14 days showed that, although vaccinated with an inactivated vaccine in presence of high levels of maternal antibodies that inhibited the antibody production, all puppies survived when challenged 4 months later with a dose of field rabies virus that killed all unvaccinated controls (Chappuis, 1998).

The successful response to vaccination in puppies from vaccinated-bitches could be explained by the level of maternal antibodies and the type of immunity response triggered upon vaccination. Indeed, except a little IgG titer (5-10%) that can be transferred transplacentally from mother to offspring, the colostrum is the main route by which maternal antibodies are transferred to puppies. Thus, the level of circulating maternal antibodies in puppies depends on the systemic immunity of the bitch and the quantity of ingested colostrum by each puppy (Chappuis, 1998). Accordingly, puppies from vaccinated-bitches may not have sufficient maternal antibodies as reported in Sri Lanka (Pimburage et al., 2017) or conversely, they may have high level of maternal antibodies which can inhibit postvaccinal production of antibodies. One of the assumptions that emerged from the study conducted in humans on determinants of infant responses to vaccines in the presence of maternal antibodies carried out by Siegrist et al.(1998), was that the B cells response was inhibited particularly when the vaccine used in infant contained the same antigen (immunodominant B-cell epitopes) as the vaccine used for immunization of his mother. However, the specific T cells response was not apparently inhibited. Thus, this study suggested the use of vaccines capable of inducing T cell

responses which, even in absence of antibody responses, could eventually contribute to protection in case of infection. Yet, it was shown that the rabies vaccine is T-cell dependent (Turner, 1976; Mifune et al., 1981; Overduin et al., 2019), providing explanation of why puppies with high level of maternal antibodies and inadequate postvaccinal antibody production survived to challenge several months later as reported by Chappuis (1998).

Above serological and challenge studies provide two evidences: (i) the satisfactory immunocompetence of puppies within their first days of life, and (ii) the efficacy of rabies vaccine in puppies of below 3 months of age in the presence or absence of maternal antibodies. These evidences strongly suggest that puppies from vaccinated or unvaccinated bitches against rabies may be vaccinated from 2 weeks of age. Furthermore, if the majority of bitches are not vaccinated against rabies, the risk of inhibition of the serological response of vaccine in puppies below 3 months of age is negligible. For more realistic practice, we suggest the vaccination of puppies at weaning (≥8 weeks of age). In addition, as a reminder (see discussion section of Chapter 3) it was suggested that a single vaccine may provide a lifetime protection against rabies given that the duration of immunity provided by rabies vaccine (≥3 years) (Lakshmanan et al.,2006 ; Schultz, 2006 ; Roth and Spickler, 2010; Malerczyk et al., 2007; Moore et al.,2015; Day et al.,2016) was longer than the life expectancy (≤3 years) of dogs in poor settings (Jibat et al.,2015). In these poor and high risk settings, due to high turnover rate (36%) as the direct results of high birth rate (45%) and high disappearance rate (40%), close to 50% of dog population is made up of puppies and juvenile dogs. When puppies are excluded from vaccination program, they are responsible for the rapid decrease of coverage, supporting the recommendation of annual mass vaccination strategy for prevention of rabies outbreaks (Fig. 3.7). Yet, when puppies are systematically vaccinated at weaning, the vaccination coverage does not fall, eliminating the need of logistically difficult and costly annual mass vaccination campaign strategy (Fig. 3.8). This result confirmed epidemiological and economic reasons of including puppies in vaccination program as suggested by some studies (Kaare et al., 2009; WHO, 2013).

5.3. How to control rabies in resource-poor settings

The study found that country such as DRC has a rabies network made up of medical and veterinary structures, despite inadequate collaboration between both sectors. This rabies network together with the community engagement must be used as framework for

implementation of an efficient and sustainable rabies control program. Therefore, approaches for improving rabies control program are discussed below.

Dog-mediated human rabies mainly affects poor areas of Africa and Asia where people are less educated and where access to prompt and appropriate post-exposure prophylaxis (PEP) is limited or nonexistent (Knobel et al., 2007; Global Alliance for Rabies Control, 2015). Yet, rabies can be eliminated at its source by vaccinating dogs, in combination with dog population management , bite management, raising public awareness and improved access to prompt PEP (WHO, 1987; Global Alliance for Rabies Control, 2015; Wallace et al., 2017). In addition, economic studies indicate that vaccination of dogs is the most cost-effective approach for preventing rabies in humans (Bögel and Meslin, 1990). Although dog-mediated rabies was successfully eliminated in some parts of the world such as Western Europe , America and the Caribbean Islands (WHO, 2013; Taylor and Nel, 2015), rabies remains endemic in several countries of Africa and Asia (Global Alliance for Rabies Control, 2015). Most of dog-rabies endemic countries are still facing barriers for rabies control, which are related to limited understanding of the local epidemiology, logistic and operational challenges, lack of resources, and competing priorities with other diseases (Wallace et al., 2017).

It is therefore important to propose an integrated, efficient and sustainable theoretical rabies control program .To achieve this goal, we used three approaches: (i) stratification of risk zones, (ii) focusing vaccination on young dogs and (iii) community engagement and One Health approach.

The first stage of reduction of resource needed for rabies vaccination program was the stratification of risk zones of rabies transmission for optimization of resource allocation by initially targeting high risk zone (Fahrion et al., 2017). The practical outcomes will be the reduction of the number of dogs that need vaccination, the number of vaccine doses and vaccination certificate, the human and logistic resources needed for massive vaccination of dog population.

The second stage is the systematic vaccination of puppies at weaning (≥8 weeks of age). Theoretically, this strategy may further reduce material, human and logistic resource needed for an effective rabies vaccination program. Indeed; with this strategy, the target population (weaned puppies) for vaccination is very low (≤10% of dog population). In addition, it is assumed that a single dose of rabies vaccine provides a lifetime protection

against rabies as discussed above. In contrast, with the annual mass vaccination campaign strategy, all accessible dogs are vaccinated irrespective of their prior vaccination status, resulting in many dogs being revaccinated every year and the high needs for resources. Yet, the revaccination recommendation is not justified given the duration of immunity provided by the vaccine (≥3 years) (Lakshmanan et al.,2006 ; Schultz, 2006 ; Roth and Spickler, 2010; Malerczyk et al., 2007; Moore et al.,2015; Day et al.,2016).

The third stage for improving the efficiency of rabies control program includes the community engagement and the intersectoral collaboration. The community engagement is referring to the involvement of community volunteers in conducting household dog ecology surveys, raising awareness about vaccination for increasing dog accessibility, dog bite prevention education, early warning of dead or killed suspect dogs, human bite exposures and suspect rabies cases. These community volunteers called “relais communautaires” are actively used in the community-based surveillance in medical health sector since 2005 in DRC (Mawazo, 2013). Their role could be expanded to above-mentioned rabies activities involving a strong collaboration between medical and veterinary sectors. In addition, the setting up of an integrated bite case management (IBCM) approach as a result of improving communication across medical health and veterinary workers, is crucial for prevention of human rabies deaths through the appropriate use of post-exposure prophylaxis (PEP) and money-saving by avoiding the overuse of costly PEP (350 USD). For purely epidemiologic reasons, data-sharing between health facilities and veterinary structures is useful for assessing the impact of implemented dog vaccination strategy. In addition, available cold chain in health facilities (PATH, 2016), may also help for storage of veterinary rabies vaccine given poor veterinary infrastructures (Ministère de Pêche et Elevage, 2017). These local resources need the legal collaborative framework for their mobilization.

The lesson to be drawn from this section is that even in resource-poor countries; human rabies incidence may be reduced by vaccinating dogs in high risk zones and appropriate use of costly post-exposure prophylaxis through a strong collaboration mainly between medical and veterinary sectors at local level and community engagement. DRC is lucky given that the country has large network of health facilities and veterinary structures, despite that the capacity building is necessary mainly for efficient use of veterinary infrastructures (Fig. 5.1.). Finally, the study provides to policy makers financially sustainable and technically efficient alternatives for rabies control in resource-poor settings.

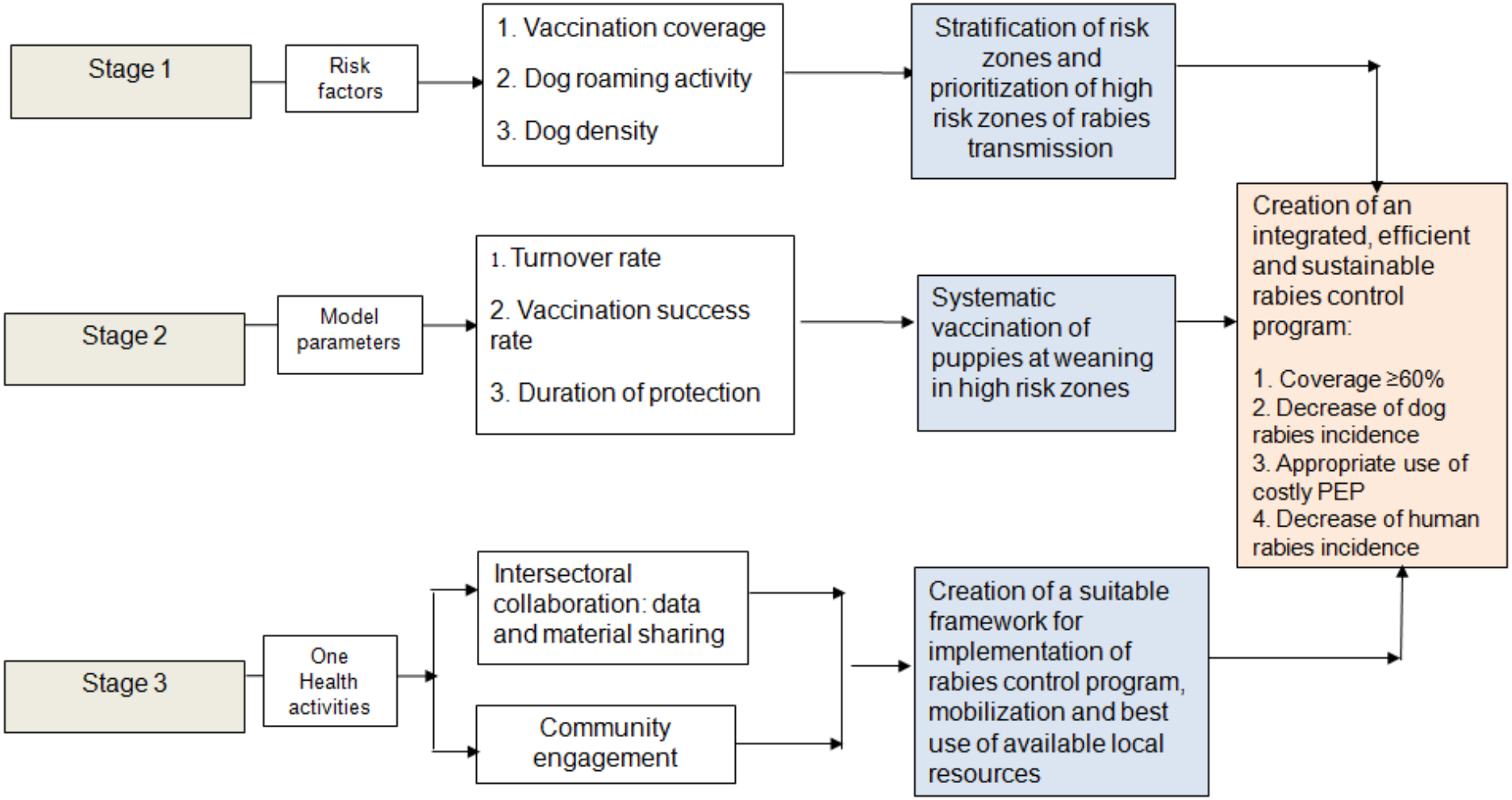

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Figure 5.1. Framework for improving the rabies control program in resource-poor settings

Chapter 6. General conclusion and perspectives

6.1. Conclusion

This thesis aimed at assessing factors of dog rabies maintenance and proposing an integrated, efficient and sustainable theoretical rabies control program in DRC.

The understanding of local risk factors is useful for designing the most appropriate rabies control program. Our study has contributed substantially to understand the risk factors of rabies transmission in urban setting. It highlighted that poor dog-keeping practices and low vaccination coverage are the main factors of rabies transmission in dog population. Both risk factors are strongly influenced by socioeconomic status of households, and consequently help to define different risk zones within urban settings. These are low, moderate and high risk zones. Usually high risk zones corresponded to poor settings. In these settings, the vaccination of dogs and the appropriate use of post-exposure prophylaxis are short-term actions for rabies control. The dog management program may be challenging by the poor dog ownership and especially the absence of physical barrier for dog roaming restriction. Accordingly, three approaches were proposed for setting up an integrated, efficient and sustainable rabies control program. The prioritization of high risk zones for rabies actions such as mass vaccination was assumed as the first way of improving the efficiency of rabies program through reduction of resource needs. The systematic and single vaccination of puppies at weaning (≥8 weeks of age) was assumed as the second way of significant reduction of need for resources. In a complementary way, the community engagement and the strong collaboration between mainly health facilities and veterinary structures, may improve the dog accessibility and therefore rapid increase of vaccination coverage, monetary saving through appropriate use of costly post-exposure prophylaxis. Finally, this study provides to policy makers financially sustainable and technically efficient alternatives for rabies control in resource-poor settings.

6.2. Perspectives

Our research hypothesized the fact that dog-mediated rabies may be controlled in resource-poor settings or countries through setting up an integrated, efficient and sustainable rabies program. It remains to be tested in the field at low scale through followings studies.

Table 6.1. Perspective field studies

N^0	Studies (referring to 4 specific objectives)	Key findings	Perspectives
1	Risk factors of rabies maintenance in dog population	• Poor dog-keeping practices and low vaccination coverage are the main risk factors if rabies maintenance in dog population • Development of a tool for local stratification of rabies risk levels	• Use of geographical tools for urbanization classification and stratification of risk zones at area-scale • Role of wildlife in rabies maintenance • Molecular characterization of rabies viruses which are circulating in animals
2.	Rabies vaccination scheme against rabies	• Assumption that a single dose of vaccine provides a lifetime protection • Systematic vaccination of puppies at weaning prevents the decline in the proportion of vaccinated subpopulation.	• Serological follow-up of dogs vaccinated at weaning in field conditions • Longitudinal follow-up of dog population dynamics during at least 3 years
3.	Intersectoral collaboration in the rabies management	• Rabies network is made up mainly of medical and veterinary institutions • Non-involvement of wildlife sector in rabies management • Inadequate collaboration between medical and veterinary sectors in relation with rabies management	• Further institutional analysis study with a wider range of stakeholders and respondents • Efficiency use of available veterinary resources • Acceptability of the integrated approach for rabies prevention and control by the medical, veterinary and wildlife workers and the community
4	Rabies control program for resource-poor and endemic countries	• Setting up of an integrated, efficient and sustainable rabies control framework	• Cost-effectiveness comparison of different vaccination strategies in field conditions

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Appendices

Appendices of Chapter 2

Figure S2.1. Factors associated to free roaming dogs in surveyed areas in Kinshasa: residency without fence or wall, attractive sites such as open market (b) and public dumps (c,d)

Table S1.1. Owners report ways for getting rid of their dogs in case of behavioral disorder, sickness or old age

Ways for getting ride	Number of answers	Percentage (%)
To sell the dog to dog meat consumers	174	35
To abandon the dog in nature away from the residency area	42	8
To send the dog to village for hunting use	36	7
Total answers	504	

Appendices of Chapter 3

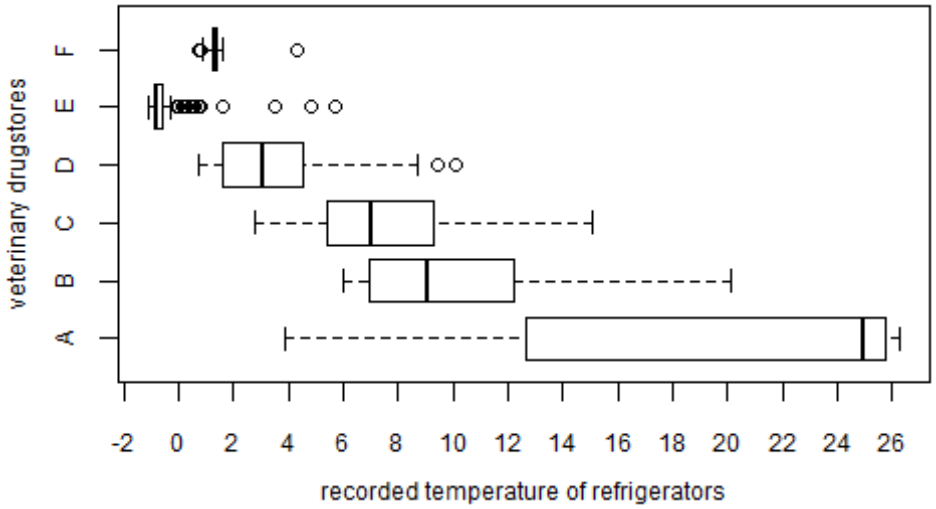

Figure S3.1. Boxplot showing overall patterns of temperature recorded in refrigerators in veterinary drugstores in Kinshasa using recording thermometer (Extech RH®) over periods of 2 days. Vaccine is assumed to be correctly stored if the third quartile of the recorded temperature is within 2 and 8^0 C.

Table S3.1. Anti-rabies antibody of puppies (2-3 months) before (D0) and after (D30) rabies vaccination

Dog	Age at vaccination (weeks)	Titer (IU/ml) at day 0 (D0)	Titer (IU/ml) at day30 (D30)	Effectiveness of vaccine in puppy
1	11-12	< 0.18	10	Yes
2	11-12	< 0.18	10	Yes
3	10-12	< 0.18	10	Yes
4	10	< 0.18	10	Yes
5	9	< 0.18	0.37	No
6	11-12	< 0.18	7.54	Yes
7	12	< 0.18	4.75	Yes
8	11	< 0.18	10	Yes
9	10	0.19	1.64	Yes
10	12	0.25	10	Yes
11	10-12	0.35	7.01	Yes
12	11-12	0.5	4.46	Yes
13	11-12	0.58	10	Yes
14	10-12	0.62	4.71	Yes
15	10-12	0.88	4.97	Yes
16	12	1.23	10	Yes
17	8	1.64	10	Yes
18	10-12	1.64	3.44	Yes
19	8	1.85	10	Yes
20	8	1.85	3.99	Yes
21	11-12	2.51	10	Yes
22	11-12	3.34	10	Yes

23	8-9	3.99	6.01	Yes
24	10	4.71	5.13	Yes

Abbreviation: IU International Unit; effectiveness criteria: D30 titer ≥0.5 if the D0 titer was < 0.5 or absolute increase of D30 titer if D0 titer ≥ 0.5

Table S3.2. Anti-rabies antibody of reported vaccinated dogs before (D0) and after (D8) booster vaccination

N^0	Time span (years) since last and booster vaccination	Titer (IU/ml) at D0 (day0)	Titer (IU/ml) at D8 (day 5-8)	Adequate anmestic response
1	1.2	0.4	5.5	Yes
2	1.3	1.71	2.26	Yes
3	1.3	0.55	10	Yes
4	1.3	0.18	5.52	Yes
5	1.3	0.15	0.61	Yes
6	1.3	0.55	10	Yes
7	1.3	4.04	5.31	Yes
8	1.3	10	10	Yes
9	1.3	10	10	Yes
10	1.5	1.71	4.45	Yes
11	1.5	1.95	10	Yes
12	1.5	0.78	0.87	Yes
13	1.5	2.06	4.96	Yes
14	1.5	0.18	0.17	No
15	1.5	1.61	10.32	Yes
16	1.6	0.18	0.39	No
17	1.8	0.52	1.25	Yes
18	1.8	10	10	Yes
19	1.9	1.09	10	Yes
20	2	1.25	5.86	Yes
21	2	4.55	10	Yes
22	2	0.51	3.81	Yes
23	2	0.48	2.28	Yes
24	2	0.51	3.67	Yes
25	2	0.31	1.55	Yes
26	2	3.12	7.04	Yes
27	2	0.32	1.84	Yes
28	2	0.31	1.65	Yes
29	2	5.18	10	Yes
30	2	1.46	5.4	Yes
31	2	1.73	1.37	Yes
32	2.5	10	10	Yes
33	2.5	2.15	10	Yes
34	2.5	5.68	10	Yes
35	2.5	1.48	10	Yes
36	2.8	1.46	5.61	Yes

37	3	7.04	10	Yes
38	3	0.53	1.52	Yes
39	3	0.53	3.06	Yes
40	3	1.19	6.46	Yes
41	3	2.89	10	Yes
42	3	0.45	1.71	Yes
43	3	0.57	1.94	Yes
44	3.5	0.35	1.17	Yes
45	4.5	0.24	1.77	Yes
46	4.5	1.34	10	Yes
47	7.5	1.11	1.57	Yes

Abbreviation: IU International Unit; adequate anamnestic response: D8 titer ≥0.5 if the D0 titer was < 0.5 or absolute increase of D8 titer if D0 titer ≥ 0.5

Appendices of chapter 4

S4.1. Check-list of key informant opinions about One Health approach implementation in rabies control in DRC

The main goal of our study is to collect your opinions on the One Health approach implementation in rabies control in DRC. I want to have a semi-structured interview with you. Any information collected from this interview will be protected and the results will be used for rabies control. Please, keep in mind that you are free to opt out of the survey. Are you willing to participate to this survey?

Date		**No.**	
Institution			
Key informant		**Key informant**	
Position		**Position**	
Phone number		**Phone number**	
E-mail		**E-mail**	
The institution role in relation with rabies			
Rabies control actions taken			

Understanding of One health (OH) approach
Involvement in OH approach and challenges encountered
One health initiatives taken in relation with in rabies management
Proposed strategies for making the OH approach a reality in the rabies management

Expectations from implementation of OH approach

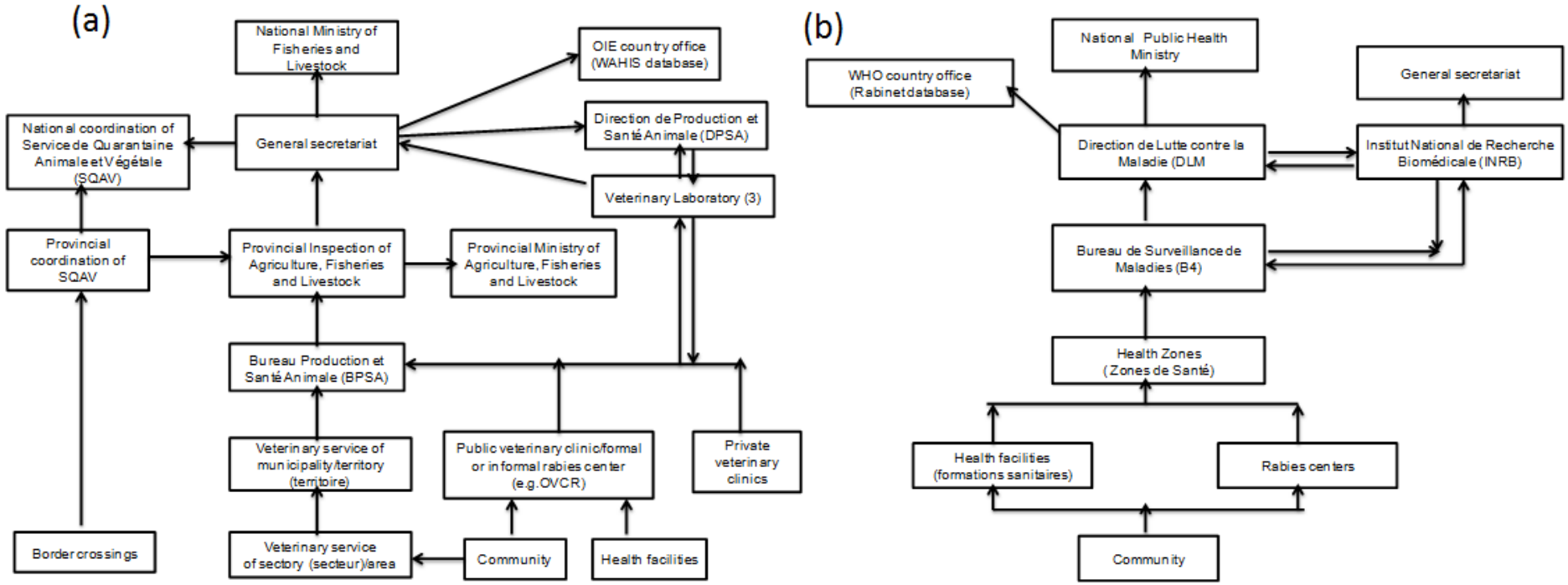

Figure S4.1. Rabies data flow in DRC. (a) Veterinary health surveillance system (Kazadi, 2014; Ministère Pêche et Elevage, 2017) and (b) Medical health surveillance system (Ministère de la Santé, 2011; Muyila et al.,2014). In both figures, the double arrow indicates the flow of samples and sample results

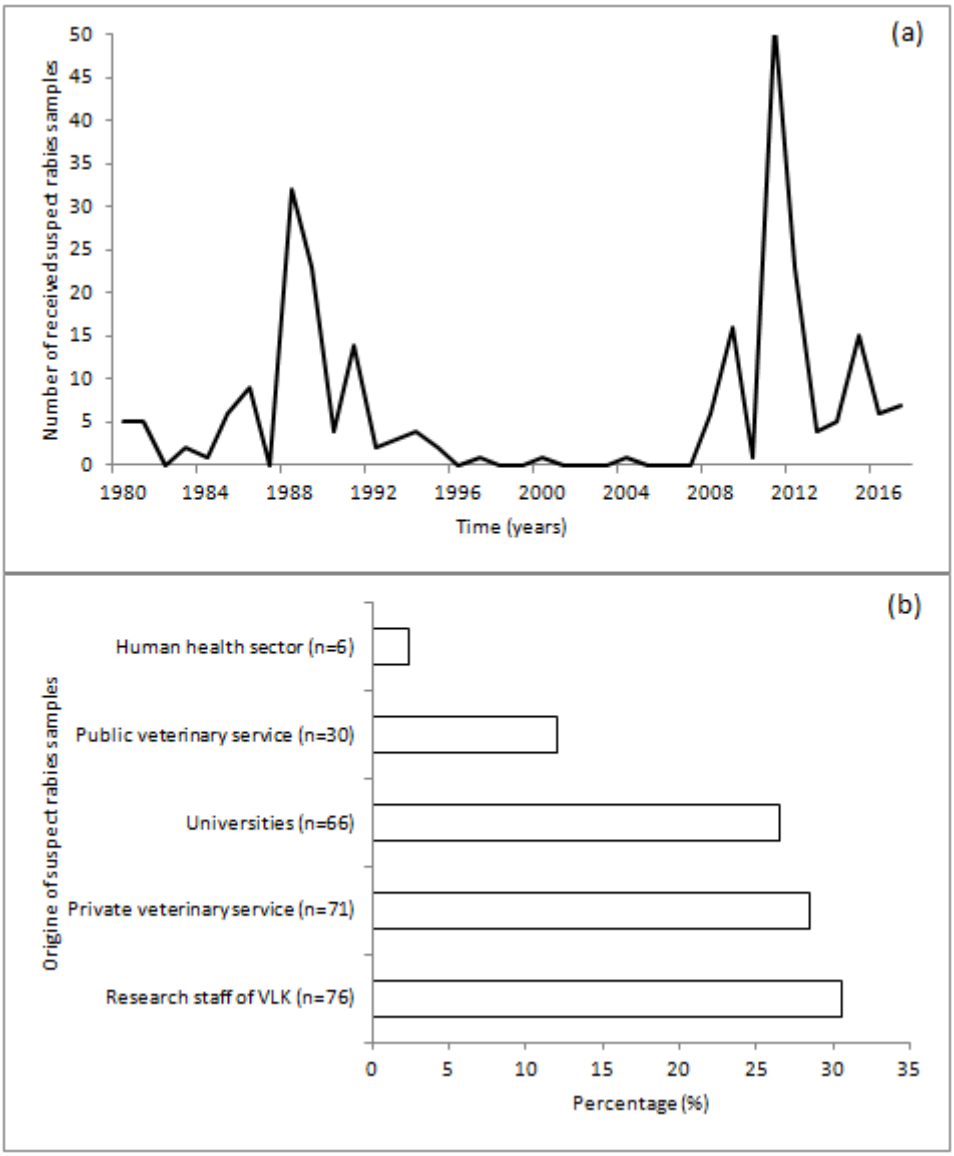

Figure S4. 2. (a) trend of suspect samples received for rabies testing from 1980 to 2017 at the Central Veterinary Laboratory of Kinshasa and (b) sample senders.